SCIENTIFIC PROCEEDINGS.

VOLUME XXVI. JUNE, 1929. No. 9.

Peping Branch

Peking Union Medical College, May 2, 1929.

4487

Some New Alkaloids from Chinese Corydalis Arbigua, Cham, et Sch. (Yen-Hu-So) Part III.

TSAN-QUO CHOU.

From the Department of Pharmacology, Peking Union Medical College.

In a previous communication[1, 2] the writer reported the isolation from the tubers of Chinese corydalis (Yen-Hu-So) five alkaloids which were provisionally labelled A, B, C, D, and E, until further confirmation. Corydalis A is identical with Corydaline in all respects and the other 4 are new. A preliminary examination of the physiological effects of Corydalis B and C by Drs. H. P. Chu and C. Pak[1] showed that Corydalis B has narcotic and local anaesthetic properties and a cardiac augmentor action. Corydalis C is apparently a cerebral stimulant. In a second communication,[3] the isolation of 3 more alkaloids, Corydalis F, G, and H, from the same Chinese Corydalis was reported. The first 2 are phenolic bases and the third, obtained only in the form of its hydrobromide, is quaternary. The present work consists of the isolation of another new alkaloid, Corydalis I, and the identification of Corydalis F and G by means of their mono-methyl ether.

Corydalis I is also a non-phenolic base, melting at 104° C. and having a specific rotation of +112.5° in alcohol. It crystallizes out from a mixture of alcohol and petroleum ether in the form of large prisms. It forms well crystallized salts; hydrogen oxalate, prisms, m. p. 185° C.; hydrochloride, silky soft needles, m. p. 236° C.; hydrobromide, silky needles, m. p. 241° C. The following preliminary physiological tests of Corydalis I were carried out by Dr. C. Pak; with doses varying from 0.19-0.35 mg. per gm. body weight subcutaneously, Corydalis I, in the form of its hydrobromide, produces in mice and rats a slight depression of the central nervous system (drowsiness) followed within one hour by severe convulsions ending in death. In frogs, the depression is followed by exaggerated reflexes and increased extensor tonus, but no convulsions. The alkaloids, Corypalmine, which is dextro-rotatory, and its monomethyl ether, d-tetrahydropalmatine, were isolated by Späth, Mosettig and Tröthaudl[4] from Corydalis tuberosa roots grown near Vienna in 1922. Corydalis F, $C_{20}H_{23}O_4N$, is similar to Corypalmine in its molecular formula and general properties, but differs from it in its optical property. Its mono-methyl ether has now been prepared by methylating with nitrosomethylurethane in the presence of sodium hydroxide. It has a molecular formula $C_{21}H_{25}O_4N$ (found: C = 70-78; H = 7-00; $C_{21}H_{25}O_4N$ requires C = 70-98; H = 7.04) a m. p. 140° C. and a specific rotation —275°. It crystallizes out from alcohol in prisms and forms well crystallized salts. The close resemblance between Corydalis F and Corypalmine on one hand and its monomethyl ether with d-tetrahydropalmatine on the other leads the writer to think that Corydalis F is probably l-Corypalmine.

---

[1] Chou, T. Q., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 544.

[2] Chou, T. Q., *Chinese J. Physiol.*, 1928, ii, 203.

[3] Chou, T. Q., *Chinese J. Physiol.*, 1929, iii, 69.

Corydalis G, when methylated in a similar way as above, gives rise to the formation of Corydaline. This proves beyond doubt that Corydalis G is Corybulbine, an alkaloid isolated from commercial Corydaline by Freund and Josephi[5] in 1893, as the latter yields also Corydaline on methylation.

---

[4] Späth, E., Mosettig, E., and Trothaudl, O., *Ber. d. Deutsch. Chem. Gesellsch.*, 1923, lxvi, 877.

[5] Freund and Josephi, *Anualen*, 1893, cclxxvii, 1.

4488

Composition of Antigen-Precipitin Precipitate.

HSIEN WU, PETER P. T. SAH AND C. P. LI.

From the Departments of Biochemistry and Pathology, Peking Union Medical College, Peping.

In a previous study[1] on the composition of the precipitate produced by the reaction of an antigen with its anti-serum, we used hemoglobin as the antigen, because we had a specific method for the determination of small amounts of this protein.

On account of the difficulty of preparing anti-hemoglobin serum, hemoglobin is not a suitable antigen for the further study of the problem. We have, therefore, looked for some other protein which is a stronger antigen than hemoglobin and which also lends itself to quantitative determination in a mixture of proteins. Iodo-albumin has been found to serve our purpose, and we will report some results which have been obtained with it.

The iodo-albumin is prepared as follows: 100 cc. of 5% sodium carbonate was added to 400 cc. of 2.5% crystallized egg-albumin solution. After cooling to 0°, the solution was mixed with an iodine solution prepared by dissolving 5 g. of iodine in 150 cc. 10% KI solution. The mixture was allowed to stand in the ice-box for 24 hours. The iodo-albumin formed was precipitated by neutralizing the solution and then adding a slight excess of acetic acid. The precipitate was washed twice with water by centrifuging. It was then dissolved in 200 cc. N/10 Na_2CO_3 to give an approximately 1% solution. It is free from iodide and iodine and has a yellow color. The iodo-albumin prepared in this way contained 14.1% nitrogen and 6.9% iodine.

Iodo-albumin is a good antigen. No difficulty was encountered in preparing the anti-serum. Rabbits were immunized by giving 3 series of injections. In each series, intravenous injection of 5 to 10 cc. of 1% iodo-albumin was given daily for 3 days. Rabbits were then rested for 3 days before another series was started. The sera were tested on the 4th day after the last injection.

The general analytical procedure used in the previous study was followed in the present work. The precipitate, after 2 washings with 0.8% NaCl, was dissolved in 1 cc. N/10 NaOH and diluted, according to the amount of the precipitate, to a suitable volume

---

[1] Wu, H., Cheng, L. H., and Li, C. P., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 853.

(5-20 cc.). Aliquot portions of the solution were taken for N and I determinations. The N was determined by the same micro Kjeldahl method used in our previous study. The iodine was determined by a colorimetric method to be described elsewhere. The amount of antigen-N was calculated by multiplying the iodine figure by 2.04.

TABLE I.

Analyses of precipitates produced by the reaction of iodo-albumin with its anti-sera.

Rabbit No.	Amount serum cc.	Conc. of iodo-albumin 1.43 mg. N per cc.	Iodo-albumin in ppt.		Total N in ppt.		Iodo-alb.-N in total -N %
			I mg.	N mg.	Total mg.	per cc. serum mg.	
911							
(a)*	8	1/30	0.0584	0.116	1.02	0.126	11.7
(b)	8	,,	0.0535	0.110	0.94	0.118	11.7
(c)	8	,,	0.0508	0.104	0.93	0.116	11.2
913	8	1/10	0.0762	0.156	1.55	0.194	10.0
	6	1/15	0.0761	0.156	1.42	0.236	11.0
	6	1/20	0.0957	0.196	1.73	0.289	11.3
	6	1/30	0.0692	0.142	1.30	0.216	10.9
	6	1/60	0.0442	0.091	1.01	0.168	9.0
914	11	1/20	0.0345	0.069	0.70	0.063	9.9
	10	1/30	0.0409	0.082	0.80	0.080	10.3
	10	1/45	0.0393	0.079	0.87	0.087	9.1
	10	1/60	0.0383	0.077	0.85	0.085	9.1
915	12	1/20	0.0829	0.169	1.69	0.141	10.0
	12	1/30	0.0885	0.181	1.77	0.147	10.2
	12	1/60	0.0738	0.151	1.57	0.130	9.7
	14	1/90	0.0653	0.133	1.52	0.109	8.8
916	10	1/10	0.1326	0.271	2.56	0.256	10.6
	6	1/20	0.0809	0.165	1.70	0.284	9.7
	6	1/30	0.0621	0.127	1.51	0.251	8.4
	6	1/60	0.0352	0.072	1.10	0.184	6.5
	13	1/90	0.0492	0.101	1.62	0.125	6.2

* (a), (b), (c) Precipitate washed 2, 3, 4 times respectively with 0.8% NaCl.

The results of some analyses are shown in the accompanying table. It will be noted that the iodo-albumin content of the precipitate is approximately constant for different sera when the concentration of the antigen used is higher than 1/60 of 1%. With lower concentrations, however, the iodo-albumin content tends to decrease.

These results confirm our findings with hemoglobin. The approximate constancy of the antigen content of the precipitate strongly suggests a chemical reaction between the antigen and the precipitin (protein), but the question cannot be decided without further study. We are planning to use horses for the preparation of large quantities of immune sera and to study the reaction of the isolated precipitin (protein).

4489

Function of the Columnar Epithelium of the Ciliary Body of Albino Rabbits.

A. PILLAT AND W. C. MA.

From the Departments of Ophthalmology and Anatomy, Peking Union Medical College, Peping.

The assumption of secretory function of the non-pigmented epithelium of the ciliary body (Leber,[1] Seidel[2]) has not been satisfactorily confirmed. Some clinical experience of quick absorption of vitreous hemorrhages, however, seems to point to a possible absorbing function; and Meller[3] was able to show blood pigments in the inner layer of ciliary epithelium in such a case. Experiments were therefore attempted on albino rabbits to investigate this question.

The ciliary body of the rabbit is covered by an inner and an outer layer of epithelium. The inner epithelial cells are not uniform, some being columnar, others cubic or flat, while those of the outer layer are uniformly cubic. Each of the columnar cells, which are concerned in this study, is limited sidewise by a very thin membrane and outwardly by a definite thick membrane closely similar to the cuticular membrane of the intestinal columnar cells. Further, the intracellular arrangement of mitochondria and Golgi apparatus is identical to that of the intestinal absorbing cells.

These structural similarities led to the following absorption tests. The foreign material was introduced into the posterior chamber of one eye, the other serving as a control. A fine needle was inserted in the upper half of the eyeball obliquely through conjunctiva and solera to prevent outflow of the injected material after removal of the needle. Two hours after injection, the lower sector of the ciliary process, *i. e.,* the sector opposite the place of injection, as well as the ciliary body of the control eye were fixed and prepared for study.

Indian ink injection: From 0.1 to 0.2 cc. Indian ink was given. Specimens were fixed in 10% formalin and stained with hematoxylin-eosin.

At the end of 2 hours, fine black granules appeared in the intercellular spaces and columnar cells. The distal portion of the latter were somewhat injured judging from the flattening of the cell and uneven staining. Occasionally ink granules were found in the connective tissue of the ciliary body, probably being carried there by wandering cells which are visible in different sections.

---

[1] Leber, Th., *Graef. Arch. f. Ophthlmol.,* 1873, xix, 87.

[2] Seidel, E., *Graef. Arch. f. Ophthlmol.,* 1918, xcv, 1.

[3] Meller, J., *Arch. of Ophthl.,* 1928, lvii, 134.

If the ink was kept 1 or 2 days in the posterior chamber, more ink granules were found evenly distributed in the epithelial cells which became eventually flattened as if the inner layer had been "shed". Even the nuclei became loaded with ink granules. It usually took 9 days for the gradual disappearance of the ink granules.

The ink granules might appear in the covering cells of the anterior surface of the iris, but very seldom in that of the posterior surface. The columnar ciliary epithelium near the *ora serrata* did not contain any ink granules, but a few were found in the chamber angle and Schlemm's canal one day after injection. The outer layer of ciliary epithelium and endothelium of the posterior surface of cornea were always free from ink particles.

Prussian blue reaction: Two drops of a fresh solution of equal parts of 1% sodium citrate and 1% ammonium ferricyanide were employed. Specimens were fixed in Carnoy's fluid and stained with haematoxylin-eosin.

The columnar epithelial cells were not injured with this injection as with the ink. The cytoplasm was stained red, containing a great number of deep blue or green granules around each of which there was a clear space. The color of the granules faded easily at the outer part of the cell. They were not found in the choroid, chamber angle or Schlemm's canal.

As the original solution injected was yellow, the presence of blue or green granules in the cell should indicate a previous absorption of the solution. The surrounding clear area may be secondary to precipitation of the granules. If the ferricyanide was kept longer in the eye, the green or blue granules would disappear, leaving behind clear spaces or vacuoles.

Fat absorption: A few drops of cod-liver oil were used. Specimens were fixed in Altman's osmic bichromate mixture or Bensley's acetic osmic bichromate mixture. Paraffin sections were stained with aniline acid Fuchsin and differentiated with alcoholic picric acid. The mitochondria were stained red, fat droplets black, nuclei yellow, and cytoplasm yellowish red.

The fat granules were found throughout the cytoplasm of the columnar cells. They might be seen in the swollen part of the filamental mitochondria and sometimes might be connected with one another in the form of chain embedded in the filaments. They were also found in large amounts in the connective tissue of the ciliary body and choroid. None has been observed, however, in the cells of the anterior iris, Schlemm's canal and retina.

The fat might disappear altogether from the ciliary epithelium 8 to 12 hours after injection, but became abundant in the choroid probably carried there by lymph streams and wandering cells.

A comparative study of fat absorption of the intestinal cell was made side by side. Mice and rabbits were fed frequently with cod-liver oil and killed after 2 to 4 hours. The mucous membrane of the stomach and intestine was removed and treated according to the same technique as used for the ciliary epithelium. The distribution of the fat granules in the intestinal absorbing cells was similar to that of the ciliary columnar epithelium. More significant is the fact that the fat granules were never found in any type of the secretory cells of the stomach and intestine.

The above experiments tend to show that the ciliary columnar epithelium has an absorbing function.

4490

Preparation of Globin.

HSIEN WU.

From the Department of Biochemistry, Peking Union Medical College, Peping.

Schulz[1] in 1898 prepared globin, the protein moiety of hemoglobin, by extracting an acidified solution of oxyhemoglobin with a mixture of ether and alcohol. Most of the pigment went into the ether-alcohol layer, leaving the globin in the aqueous layer. Upon neutralization with ammonia the globin was precipitated which was redissolved in dilute acetic acid and dialyzed. This method is very convenient, but the globin thus obtained is not natural, but denatured, globin, since the ether and the alcohol rapidly denature the protein in the presence of the acid.

Hill and Holden[2] have recently succeeded in preparing some globin which is soluble in neutral solution and which may be natural globin. They did not start with pure hemoglobin, but with washed cells. They observed that the stroma proteins, when swollen with ether, had a great power of absorbing hematin, and they utilized this property for the removal of the pigment from the globin. Cell solution was acidified with HCl and shaken with ether and kieselguhr. The mixture was filtered, and the filtrate was neutralized with ammonia to precipitate the denatured globin. This was again filtered, leaving a soluble globin in the filtrate. To minimize denaturation, all these operations were carried out at —2°.

---

[1] Schulz, F. N., *Z. Physiol. Chem.*, 1898, xxiv, 449.

[2] Hill, R., and Holden, H. F., *Biochem. J.*, 1926, xx, 1326.

As far as the separation of the pigment from the globin is concerned, the extraction method cannot be surpassed. The stroma proteins remove only a part of the pigment, the remainder being carried down by the denatured globin. Hill and Holden stated that the globin prepared by their method contained up to about 2% of methemoglobin. It would seem that the globin solution may be contaminated also by the stroma proteins. Working with small amounts of cells and "with scrupulous attention to details," Hill and Holden reported a yield of globin amounting to about 40% of the original hemoglobin. Working at 0° instead of —2°, and using the cells of sheep instead of ox blood we have been able to obtain a yield of only a few per cent.

The commendable feature of Hill and Holden's method is the cooling of all solutions. We have found that if the extraction of the pigment with ether and alcohol is carried out in the cold and the denatured globin removed by neutralization and filtration, considerable amount of globin is found in the filtrate. As the result of a certain finding about the hemoglobins and guided by our experience with denaturation of proteins in general, we have worked out a simpler method for the preparation of globin.

Method: Prepare methemoglobin as follows: Dilute 100 cc. of washed sheep cells with an equal volume of H_2O. Add 4 g. of powdered potassium ferricyanide. When the evolution of oxygen has ceased, transfer the mixture to collodion tubes and dialyze until free from ferricyanide. The dialyzed solution is treated with alumina cream to remove the stroma proteins.

Measure (a) 32 cc. of 95% alcohol and 112 cc. of ether into a 250 cc. separatory funnel; (b) 150 cc. of water into a 300 cc. flask; (c) 20 cc. of N/10 NaOH into a test tube. Cool all these to 0° by placing them in an ice bath. Mix 40 cc. of 2% methemoglobin solution with 20 cc. of N/10 HCl and cool the mixture also to 0°.

Pour the acid methemoglobin solution into the ether-alcohol mixture. Remove the separatory funnel from the ice bath and shake vigorously for 30 seconds. Return the funnel to the bath. In one or two minutes the mixture will have separated into 2 layers. The upper layer contains nearly all the pigment, while the lower layer contains the globin. Remove the funnel from the bath and deliver the aqueous layer into the cold H_2O (b) and mix. Pour the N/10 NaOH into the mixture and mix by a gentle shaking. The denatured globin is precipitated. The precipitate flocks well and is very easily filtered. The filtrate contains the globin in a concentration of about 0.2%. If the ether-alcohol mixture is cooled to —15° instead of 0°, the yield is a little higher. The yield is also higher, if 1% methemoglobin solution is used instead of 2% solution. In that case, the final solution of globin is, of course, more dilute.

To remove the ether and alcohol, the solution is dialyzed against water in the cold. If it is desired to concentrate the solution at the same time, the globin solution may be dialyzed against egg white as described in the following paper.

Discussion. In the preparation of globin Schulz used oxyhemoglobin and Hill and Holden used washed cells which contained, of course, also oxyhemoglobin. We have found that different forms of hemoglobin do not give up the pigment to the ether-alcohol mixture with the same ease. Whereas a 1% solution of methemoglobin gives up all of its pigment when extracted as described above, oxyhemoglobin gives up only about 80% of its pigment under the same conditions. If the aqueous layer in the latter case is shaken with ether-alcohol mixture for a second time, very little pigment is removed, and further extraction will not remove any more pigment. If the oxyhemoglobin is reduced in Van Slyke's gas apparatus (constant pressure form) and the extraction carried out in vacuum, the pigment is completely extracted as in the case of methemoglobin. Carboxyhemoglobin occupies a middle position between the methemoglobin and the oxyhemoglobin. This difference in the behavior of different forms of hemoglobin seems significant, and it will be studied further. For the present it suffices to point out the fact that methemoglobin is the most suitable form of hemoglobin for the preparation of globin.

For the complete extraction of the pigment a certain minimum amount of acid is required. Excess of acid is not desirable, as it will accelerate the denaturation of globin. The amount prescribed in the above method is just enough for complete extraction of the pigment from 1% methemoglobin solution. When 2% methemoglobin solution is used, a small amount of the pigment is left in the aqueous layer. The pigment is carried down, however, by the denatured globin upon neutralization of the acid solution.

The dilution of the acid globin solution with H_2O before neutralization is to decrease the denaturing action of alcohol and ether which are dissolved in the solution.

When a solution of hem (prepared by dissolving hemin crystals in dilute sodium carbonate) is added to an equivalent amount of globin and the mixture is buffered to pH 7.4, the absorption band between C and D ($\lambda = 634$) characteristic of methemoglobin can be seen. In 2% Na_2CO_3 solution the 3 bands of alkaline methemoglobin mentioned by Hill and Holden can be seen. When compared with a natural methemoglobin solution of the same concentration, however, the intensity of the bands given by the globin-hem mixture is very much weaker. In fact, the difference in color between the methemoglobin and the globin-hem mixture in carbonate solution is apparent to the naked eye. The globin solution prepared according to Hill and Holden behaves in the same way as one prepared by our method. We are not prepared to interpret this finding at present, and we are studying further the relationship between hem, globin and methemoglobin.

4491

Method for Concentrating Protein Solutions Without Denaturation.

HSIEN WU.

From the Department of Biochemistry, Peking Union Medical College, Peping.

In the preparation of natural globin by the method described in the preceding paper the solution obtained is too dilute for certain purposes. It has been found impossible to concentrate the solution by any of the usual methods without great loss as the globin is very susceptible to denaturation by a variety of agents. Finally a method is found which works well for globin and should be applicable to any protein solution.

The principle of the method is simple, but it does not seem to have been employed as a laboratory technique. The protein solution is concentrated by dialysis against a concentrated solution of another protein or some other substance which cannot diffuse through the membrane. Fresh egg white serves this purpose very well. It is convenient to insert into the tube a rubber stopper with a large hole. The tube is tied to the stopper with a string which serves for hanging the tube.

A collodion tube such as is used for dialysis is prepared. Transfer to the tube a measured volume of water and place the tube in about 10 volumes of fresh egg white. The dialysis is allowed to take place in the ice chest.

After 24 hours the water in the tube will have decreased to a small fraction of the original volume. This water is removed with a pipette and tested for protein. If the test is negative, the collodion tube is suitable for use.

The dilute protein solution to be concentrated is transferred to the tube and dialyzed against egg white. As the solution becomes concentrated more of the dilute solution is added to it from time to time. The dialysis is continued with frequent change of the egg white until the desired concentration is reached. If it is necessary to remove the crystalloids which have come into the solution from the egg white, this can be accomplished by dialysis under pressure against distilled water in the usual way.

4492

Behavior of Adrenal Transplants in the Transparent Chamber Inserted into the Rabbit's Ear.

HSIANG-CH'UAN HOU. (Introduced by R. K. S. Lim.)

From the Department of Physiology, Peking Union Medical College, Peping.

Adrenal transplants were made into the ears of 6 adult rabbits by means of Sandison's technic.[1] The left adrenal gland was removed aseptically from the rabbit and placed in warm saline for 10 to 15 minutes. Thin pieces of both cortex and medulla were taken and inserted into the chamber adjacent to the blood vessels. Immediate observation showed that the transplant consisted of cell masses too opaque to be clearly seen, with a few isolated cells at the edge. These individual cells appear to live for 2 to 4 weeks, exhibiting some migrating activity but never active amoeboid movement. They are quite large and show vacuoles and a number of highly refractile granules. In 4 days to a week the vacuoles became more numerous and many of the cells began to shrink, fragment and disappear. No cells were seen to be growing and multiplying.

The main transplant showed some living cells along the margin but the center when visible appeared to be necrotic and became slowly absorbed in the course of about 4 weeks.

The transplant apparently retards the growth of the connective tissue and blood vessels in the chamber. No adrenal cells were seen to live in among the connective tissue, nor was there any indication of reaction about the transplant, except for the presence of a few leucocytes and macrophages. Occasionally a zone of leucocytes, 3 or 4 cells deep, was found next to a degenerating mass.

---

[1] Sandison, J. C., *Am. J. Anat.*, 1928, xli, 447.

All 6 rabbits, 5 with autotransplant and one with hemotransplant showed quite similar findings. None of the transplants exhibited signs of growth or survived longer than 6 weeks.

Attempt was also made to transplant the adrenal in 2 stages. The transparent chamber was first inserted into the ear of the rabbit. When the growth of blood vessels became profuse throughout the greater portion of the chamber, a second operation was made to remove one adrenal, thin pieces of which were inserted into the chamber. There was no hemorrhage.

In the first few days the blood vessels and connective tissue did not show any reaction towards the graft, which appeared to draw its nourishment from the lymph in which it was bathed. On the third day, the capillaries increased in number with a more rapid flow of blood through them and some extravasation of red blood corpuscles occurred. More leucocytes were seen on the fifth and sixth days, although phagocytosis was not actually observed.

In the week following, regeneration of the transplant was noted to go on hand in hand with degeneration. Most of the cells in the center of the transplant degenerated rapidly, while cells along the edges continued to grow. Mitotic figures were only rarely seen. Columns of cortical cells were now visible, separated from the main transplant, some presenting the typical appearance of the fascicular zone of the adrenal gland. Daily measurement with an eye-piece micrometer showed that these columns increased, *i. e.,* grew, in length for a week or two. Medullary cells must have degenerated, for none were seen.

By the third and fourth week, the graft became canaliculized with blood vessels and a number of columns of cortical cells became easily visible. The cells were rendered clearer after subcutaneous injection with neutral red according to the technic described by W. C. Ma and H. C. Chang,[2] although the cell granules were not distinctly stained. In the fifth week, however, a retrogression in the growth appeared and most of the columns became broken up into segments and individual cells, which degenerated and were gradually replaced by connective tissue cells. By the twelfth week only a few scattered cells and a few columns remained.

Jaffe[3] records that his adrenal cortex transplants (into abdominal

---

[2] Ma, W. C., and Chang, H. C., *Chinese J. Physiol.*, 1928, ii, 381.

[3] Jaffe, H. L., *J. Exp. Med.*, 1927, xli, 587.

wall of guinea pigs) were still viable after 276 days; Busch, Leonard and Wright[4] that segments of whole adrenal gland implanted in the kidney (rabbit) survived for 274 days. Pybus[5] and Leschke[6] report successful homotransplantation in humans with Addison's diseases; the grafts apparently lived for a year or longer. All other attempts at transplantation have more or less failed.

The results here described show that an implant will only grow so long as the growth of connective tissue is held in check.

Jaffe suggests that the presence of medullary tissue in the graft prevents successful takes.

---

[4] Busch, F. C., Leonard, T. M., and Wright, T., *J. Am. Med. Assn.*, 1908, li, 640.

[5] Pybus, F. C., *Lancet*, 1924, 550.

[6] Leschke, E., *Med. Klin.*, 1928, xxiv, 1268.

Southern Branch

Tulane University, May 23, 1929.

4493

Parasiticidal Potencies of the Tri- to Hepta-Methyl Derivatives of Rosanilin.

ERNEST CARROLL FAUST.

From the Laboratories of Parasitology, Peking Union Medical College, and Tulane University.

Faust and Yao[1] have shown that gentian violet, either by oral or by intravenous injection, is parasiticidal for *Clonorchis sinensis,* a fluke residing in the biliary passages of man, dogs and cats in the Far East. Although in chronic cases of long standing the flukes are at times so effectively walled off by the thickening of the biliary epithelium that it is impossible for the dye to reach them in lethal amounts, from 60 to 95% of the flukes can be destroyed by this dye, thus producing material improvement in the patient. Olivier and Kandou,[2] utilizing gentian violet therapy on 18 Clonorchis-infected patients in Java, have been able to confirm the results of Faust and Yao. It has seemed desirable, therefore, to follow the experimental work a step further in order to determine (1) which homologs in the methylated rosanilin series possess the parasiticidal properties and, if possible, (2) the portion of the potent homologs which is responsible for the results. With this in view the following dyes were obtained and the bactericidal and clonorchicidal potencies tested.

1. Dahlia crystals, *e. g.,* tri-methyl rosanilin (National Aniline and Chemical Co., and Grübler).
2. Gentian violet crystals, *e. g.,* a half and half combination of tetra-, penta-, and hexa-methyl pararosanilins with dextrin. (N.A. C.C.).

---

[1] Faust, E. C., and Yao, K. F., *Arch. fur Schiffs- u. Tropen-Hygiene,* 1926, xxx, 383.

[2] Olivier, P. H., and Kandou, R., *Geneeskundig Tyschrift voor Nederlandsch-Indië,* 1927, lxvii, 59.

3. Enteric coated gentian violet pills (N.A.C.C.).

4. Methyl violet B crystals, *e. g.*, about 8% tetramethyl, 80% penta-methyl and 12% hexa-methyl pararosanilin. (N.A.C.C.).

5. Crystal violet crystals, *e. g.*, pure hexa-methyl para-rosanilin. (N.A.C.C.).

6. Methyl green, *e. g.*, hepta-methyl pararosanilin. (Grübler.)

Table I shows the relative bactericidal potencies of the several dyes at different dilutions.

TABLE I.
Growth of B. subtilis in the following media.

Dilution	1 Dahlia Nat. An. Chem. Co.	2 Dahlia Grübler	3 Gentian Violet Tablets Nat. An. Chem. Co.	4 Gentian Violet Crystals Nat. An. Chem. Co.	5 Methyl Violet B. Nat. An. Chem. Co.	6 Crystal Violet Nat. An. Chem. Co.	7 Methyl-Green Grübler
1/100,000	—	—	+	—	—	Not tried	+++
1/200,000	—	—	++	—	—	Not tried	+++
1/500,000	++++	+++	++++	+	+	—	++++
1/1,000,000	++++	+++	++++	+	+	—	++++

It will be seen that crystal violet is the only member of the series which conforms to the biological specification of the Commission on the Standardization of Biological Stains (Conn[3]), namely that it should have "sufficient bacterostatic power so that, when added to nutrient agar in the proportion of one part to a million, it will entirely prevent the growth of *Bacillus subtilis* when this organism is streaked over the surface of the hardened agar."

Guinea pigs and puppies artificially infected with Clonorchis and cats naturally infected with the fluke were used in testing the clonorchicidal potency of the dyes. Both oral and intravenous routes of administration were utilized, although the latter (in one-half per cent solution) was better tolerated by the animals. The experiments, which extended over a period of nearly a year, may be summarized as follows: 1. Dahlia was found to be without measurable parasiticidal potency or without any toxic effects on the host. 2. Gentian violet, methyl violet and crystal violet were equally potent (being 50 to 100% clonorchicidal, depending on whether the infection was chronic or of recent origin). These dyes were well tolerated by the infected animals, particularly when intravenous injection was utilized. 3. Methyl green was found to be without measurable clonorchicidal potency. However, it had a definite toxic effect on all animals to which it was administered even in small amounts. The symptoms which developed some days after administration consisted of spastic paralysis and epileptiform seizures, terminating fatally.

---

[3] Conn, H. J., *Biological Stains*, Geneva, N. Y., 1925, 151 pp.

The results of these experiments seem to indicate that the tetra-, penta-, and hexa-methyl pararosanilins are equally clonorchicidal, although theoretically, as well as by the bactericidal test, crystal violet, the pure hexa-methyl pararosanilin, should be the most potent member of the series. On the other hand the lower and higher homologs of the series, tri-methyl and hepta-methyl pararosanilins, are non-clonorchicidal, while the latter is definitely responsible for fatal disturbances of the central nervous system. The structural formulae of these dyes indicates that the compound is lethal for Clonorchis when there are no fewer than 4 and no more than 6 methyl groups. Apparently the potency is as great with 4 as with 5 or 6 such substitutions.

4494

Ineffectiveness of Radiant Energy on Hemorrhagic Anemia in Dogs.*

HENRY LAURENS AND H. S. MAYERSON.

From the Laboratory of Physiology, School of Medicine, Tulane University of Louisiana, New Orleans.

Twenty white animals in which the Hb level had been reduced by 3 or 4 successive hemorrhages, at from 2 to 3 days intervals to between 35 and 50%, were fed the standard Whipple (salmon bread) diet and irradiated with the flaming C ("Pan Ray") arc. The interval between successive exposures, as well as their number and intensity, was varied in different experiments and in successive periods of the same experiment. The efficacy of the experimental procedure in effecting regeneration was measured by the amount of Hb which had to be removed during the experimental period, as compared with that removed during earlier and later control periods, in order to keep the Hb at the chosen reduced level.

Such irradiation produces a marked increase in the number of erythrocytes which progressively decrease in size but maintain a normal saturation, as was earlier reported for "normal" animals.

---

* Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.

The eventual decrease in red cell number during the later part of the post-irradiation period is accompanied by a reciprocal increase in the size. A pronounced increase in the percentage of reticulocytes usually precedes that of the total red count. In 5 experiments the reticulocyte percentage is double, or more than double, that preceding irradiation. Massive exposures (75 to 133 g. cal. per sq. cm.) given at long intervals are usually more effective than are smaller doses repeated more often.

An increase in Hb was noted in only one experiment. In 11 cases the amount of Hb formed during and immediately following the irradiation period was less than in previous or later control periods. In the remaining 8 cases there was no effect. It is to be emphasized that dried peaches, apricots, or lettuce added to the diet of animals which had shown no response to radiation produce their typical influence, although the accelerated Hb regeneration produced by adding these substances to the standard diet was not demonstrably influenced by any dosage of radiant energy. It is further of interest that the changes in reticulocytes and erythrocytes which characteristically follow flaming C arc irradiation of dogs on the standard bread diet are now absent. Irradiation of 6 dogs with the quartz Hg vapor arc (Cooper-Hewitt) gave similar results.

4495

Ineffectiveness of Radiant Energy on Anemia in White Rats.*

PAUL C. FOSTER. (Introduced by Henry Laurens.)

From the Laboratory of Physiology, School of Medicine, Tulane University of Louisiana, New Orleans.

Young rats placed on a milk diet (12% solution of Klim in distilled water) at about 21 days of age, grow about as well as rats do on the stock, Sherman B, diet and exhibit no evidences of avitaminosis. In 3 to 6 weeks the Hb falls to 4 or 5 gm. per cent, and the red cells to 4 or 5 million in contrast to the usual values at this age of 13 gm. per cent and 8 million. Control experiments indicate that a more severe anemia can be produced by fresh dairy milk. These anemic rats will not breed. Litters have been procured by transferring females to the stock diet and mating them with males that have always been on this diet. When the young are about 21

---

* Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University

days old they are similarly put on the milk diet. The Hb per cent and the number of red cells decrease more rapidly than they did in the first generation. When the low levels are reached some of the animals die, but the majority show a tendency to a slow spontaneous recovery. Hb values of less than 4 gm. per cent are fatal in the majority of cases.

The red cells in this type of anemia vary considerably in size and shape and many of them show polychromatophilia. The average corpuscular volume is 35 cu. microns as compared with 51 cu. microns for the normal red cell, and the saturation is low, 30% as compared to 40% in the normal cell. During the spontaneous recovery the stroma increases more rapidly than the Hb so that in the later stages the saturation is further diminished. An increased resistance of the red cells to hypotonic solutions is a striking and constant feature of this anemia.

Measured doses of radiant energy from quartz mercury vapor and flaming C arc lamps in various quantities and with varying time intervals between them were administered. In some experiments the irradiation was begun as soon as the animals were placed on the milk diet; in others not until a severe anemia had developed. The following groups served as controls: irradiated rats on the stock diet, non-irradiated rats on the stock diet, and non-irradiated rats on the milk diet. The differences in Hb and in red cells in the irradiated and non-irradiated groups were small, being slightly higher in the irradiated groups. The irradiated animals often present a more healthy appearance, and any slight increase in blood levels is referred to an improvement in the general nutritional state of the animal, rather than to direct action on the hemopoietic system. Excessive irradiation in some cases retarded growth but was not observed to have any detrimental effect on the blood.

4496

Action of Bacteriophage upon Production of in vivo Prepared Toxic Substance of Bacillus Typhosus.*

WILLIAM H. HARRIS AND OGILVIE M. LARIMORE.

From the Department of Pathology, Tulane University School of Medicine.

In a previous communication[1] we reported the results of the inoculation of guinea pigs with the toxic material obtained from the

---

* Aided by a grant from the David Trautman Schwartz Research Fund of The Tulane University.

[1] Harris, W. H., and Larimore, O. M., *J. Exp. Med.*, 1928, xlviii, 885.

exudate of experimental typhoid peritonitis. The filtered toxic material produced lesions closely simulating those of human typhoid fever and the animals died after 3 or 4 inoculations. The toxic substance produced in this manner was found to be of rather low potency. Single injections of from 3 to 5 cc. failed to produce death although the animals became sick and showed marked reactions to the inoculations.

It was thought that the employment of typhoid bacteriophage in conjunction with this *in vivo* method may serve the purpose of yielding a more toxic substance. It has already been shown that the action of bacteriophage *in vitro* has but little if any effect upon the toxicity of certain microorganisms. It was, however, thought that since the toxin employed previously by us was prepared *in vivo*, the presence of this lytic agent in the field of conflict between the host and the invading microorganism might provoke the liberation of a poison of more highly toxic nature.

Forty white mice and 24 guinea pigs were employed in the experiments. Typhoid culture 7D, a stock strain which had produced an accidental typhoid infection in one of us (Larimore) approximately a year ago and had been recovered from the blood stream, was employed for the inoculation. The typhoid bacteriophage used consisted of 2 separate "strains" kindly sent to us by Dr. D'Herelle and Dr. Bronfenbrenner.

Three sets of experiments were conducted: Series I consisted of the production of typhoid peritonitis in the mouse with the presence of varying amounts of phage introduced simultaneously with the infecting microorganism. The exudate was procured and filtered and this filtrate inoculated intraperitoneally into the other mice. Control animals in which no phage was introduced were run parallel and the filtered exudate injected intraperitoneally into other mice in similar doses to the previous animals. While the animals became sick they did not die from the single injection.

In Series II, guinea pigs were inoculated intraperitoneally with doses of 2, 3, 4 and 5 cc. of typhoid culture 7D and varying amounts of the bacteriophage were introduced one hour before, simultaneously and one hour after the infecting typhoid injection. Control animals without phage were employed. From these animals, dead of typhoid peritonitis, the peritoneal exudate was procured and filtered. These filtrates were inoculated intraperitoneally in amounts varying from 1 to 5 cc. In 4 of the animals in which phage had been used in the production of toxin, marked toxic effect as shown by twitchings and transient partial paralysis of the posterior extremities were noted. These effects were transient and the animals recovered over night. The remaining animals including the controls showed loss of activity and were manifestly sick but were lively after 24 hours

In Series III, guinea pigs were inoculated in a similar manner as in Series II but the recovered filtrate material was inoculated into white mice. In this manner comparatively large doses could be used for a very small animal but the results obtained did not indicate any difference in degree of potency of toxin in the exudate procured from animals with and without phage.

A group of animals, both white mice and guinea pigs, were inoculated with fatal doses of typhoid suspension and another group with phaged doses of typhoid bacilli. In these animals no differences in the period of time of death production was noted. It should be stated that the phaged suspension in these latter experiments was not filtered, whereas, in the other 3 series of experiments the bacteriophage employed was always filtered and tested for sterility before it was employed.

These experiments indicate that the employment of typhoid bacteriophage in conjunction with our previously reported process, has no significant effect upon the potency of toxic substance procured from the typhoid bacillus through this means.

4497

Action of Bacteriophage in Experimental Typhoid Peritonitis.*

O. M. LARIMORE AND W. H. HARRIS.

From the Department of Pathology, Tulane University School of Medicine.

While engaged in certain other experimental work, we had the opportunity to observe the effect of *Bacillus typhosus* bacteriophage upon peritonitis produced in guinea pigs and white mice, by means of the intraperitoneal injection of *Bacillus typhosus.* In view of the apparent possibilities of bacteriophage therapy, it was thought that the observations herein noted in the experimental animals, should be recorded.

A race of bacteriophage virulent for *Bacillus typhosus* was used. which was isolated and kindly furnished us by Professor D'Herelle and which in his hands had been found potent or virulent for several heterologous strains of typhoid bacilli.

---

* Aided by a grant from the David Trautman Schwartz Research Fund of The Tulane University.

The same strain of *Bacillus typhosus* was used as is described by us in the accompanying article.

At no time were we able to prevent the occurrence of secondary cultures at the end of from 48 hours to 4 or 5 days. A test of the virulence of secondary cultures was made by employing the last tube in a series of 12 dilutions, in which recurrent growth had occurred after lysis had been apparently complete for 5 days. The contents of this tube, at that time, when inoculated intraperitoneally, killed a full grown guinea pig in the same number of hours as did the suspension of the living organisms, used as a control.

To obviate the confusion resulting from the employment of such a phage, all bacteriophage was filtered, tested for sterility and pathogenicity, before inoculation.

The animals used consisted of 36 guinea pigs, and as many white mice.

The first series of experiments were performed to ascertain whether or not the *in vivo* presence of typhosus bacteriophage might prevent the formation of peritonitis. Four guinea pigs were given, intraperitoneally, 2, 3, 4, and 5 cc., respectively, of a salt solution suspension of a young culture of *Bacillus typhosus* and simultaneously there was administered, by the same route, 5 cc. of *Bacillus typhosus* bacteriophage, potency of 10-9, in ordinary bouillon of pH 7.6. A control pig receiving 5 cc. of the culture alone died in 7 hours and another receiving 2 cc. of culture alone died in 24 hours. The treated pigs in this group remained alive and healthy.

We repeated this experiment under as nearly identical circumstances as possible to determine whether this apparent protection was constant. Ten guinea pigs were treated as follows: 6 guinea pigs were inoculated in the same manner as in the first group, with similar controls. In addition 2 pigs were given 5 cc. and 2 cc. of the microorganism plus a simultaneous intraperitoneal injection of ordinary nutrient bouillon, and 2 others, the living microorganism plus 5 cc. of the same culture killed by heat. The apparent protection obtained in the first group was not confirmed, the animals dying the following day and in the same number of hours as did the culture controls, including the 2 animals which received the killed culture of *Bacillus typhosus*. Only the 2 receiving the live microorganisms and simultaneously the nutrient bouillon, remained alive and well.

In the next series of 8 pigs, 4 were injected with 5 cc. (lethal dose) of a salt solution suspension of young living typhoid bacilli intraperitoneally, and 4 with the microorganism plus typhoid bacillus bacteriophage (5 cc.). All of the animals died in approximately

12 hours. The peritoneal exudate present in the peritonitis of the animals that received only the suspension of typhoid bacilli was scanty in amount, highly albuminous, and contained myriads of non-motile bacilli and but few cells; whereas in the pigs inoculated with both bacilli and phage the exudate was copious, serosanguinous in character and contained fewer bacilli but these bacilli were very actively motile.

In order to compare more definitely the relative numbers of living bacteria present in the peritoneal exudate of the animals which had received phage with those inoculated with typhoid culture alone, the respective materials from each group were plated. The pure materials and dilutions of 1-10, 1-100 and 1-1000 were used. A very heavy growth was obtained for all plates but the exudate in which phage had been used showed a distinctly greater number of colonies. It is apparent that many of the bacilli noted by smears and hanging drop in the exudate of the pigs receiving culture alone, were dead. No evidences of lysis or increased phagocytic activity could be detected as the result of the use of bacteriophage.

In another group of animals, the phage was administered an hour before, simultaneously, and an hour following the introduction of the microorganisms. In addition certain animals injected with typhoid bacilli in suspension, were given repeated large doses of bacteriophage at hourly intervals for several hours. The results presented no distinctive differences between the control and treated animals. No evidences of protection or amelioration of the infection were observed in the animals receiving bacteriophage.

Similar experiments were also performed upon white mice. It was thought that since the lethal dose for the animals would approach more closely the direct quantitative relationship to the number of microorganisms susceptible to lysis *in vitro*, greater protection might be procured.

The procedure employed was, in general, the same as that for the guinea pigs with the exception of the smaller dosages. It was found that an injection of 0.1 cc. of the same suspension used for the pigs, proved fatal to the mice in from 7 to 8 hours. The results obtained in the mice were the same as those in the guinea pigs.

In these experiments, it is evident that the employment of *Bacillus typhosus* bacteriophage by this method, played no important rôle in the prophylaxis, production, or therapeusis of experimental typhoid peritonitis in either guinea pigs or white mice.

4498

Effect of Carbon Arc Radiation on Resistance of Rachitic Rats to Tetanus Toxin.*

S. N. BLACKBERG. (Introduced by J. T. Halsey.)

From the Department of Pharmacology, Tulane University School of Medicine.

That sunlight increases the resistance of rachitic rats to specific infections has been shown.[1, 2] In view of the known variations, daily and seasonal, in solar energy, it seemed desirable to study the effects of a more constant source of radiant energy.

The flaming C arc (Pan Ray), which has been used in this work when operated at 25-28 A, 55-60 V emits a total energy of 0.797 g. cal. per sq. cm. per minute incident at a distance of 1 meter, which is distributed as follows: ultraviolet 12, luminous 35, and infra-red 53%.[3]

The animals used were albino rats of the uniform Wistar Institute strain. The members of the different litters were evenly distributed among the test and control groups. All the animals received the same rachitogenic diet (McCollum 3143) for 50 days. During this period, Groups I, III and IV were irradiated daily for 20 minutes at 1 meter distance, thus receiving 16 g. cal. per sq. cm. This amount of radiation was sufficient to prevent the development of rickets in rats receiving the rachitogenic diet. After 50 consecutive irradiations the animals were skiagraphed to determine that there were no rachitic bone lesions, and were then injected with varying amounts of tetanus toxin (Table I). Control groups II and V received similar injections of toxin but were not irradiated. Roentgenograms of these rats showed marked rachitic lesions.

The toxin solutions were injected into the muscles of the thigh and were always freshly prepared from a stock of dried tetanus toxin. It is shown in Table I that the resistance to tetanus-toxin of the irradiated rats was much greater than that of the non-irradiated controls.

Observations on work being performed in this laboratory, although not final, suggest that C arc irradiation for 20 minutes daily at 1 meter, increases the susceptibility of normal rats to tetanus toxin.

---

* I wish to express my gratitude to Professor Henry Laurens for his helpful suggestions and for the use of the arc.

[1] Robertson, E., *Can. Med. Assn. J.*, 1927, xvii, 1033.

[2] Brown and Tisdall, *Can. Med. Assn. J.*, 1927, xvii, 1425.

[3] Laurens, H., and Mayerson, H. S., *Radiology*, 1929, xii, 328.

TABLE I.

Rat	Wt. gm.	Irradiation 20 min. 1 meter	Rachitogenic Diet	Tetanus Toxin mg. per 100 gm. Rat.	Remarks.
I-341	54-90	50 days	50 days	.005	Slight local tetanus. Recovery.
I-342	60-98	" "	" "	.01	Severe local tetanus. Recovery.
I-343	58-82	" "	" "	.005	Slight local tetanus. Recovery
I-344	60-70	" "	" "	.01	Severe local tetanus. Recovery.
I-345	26-44	" "	" "	.02	Died—5 days after injection.
II-346	44-	0			Died. Severe rickets.
II-347	48-80	0	" "	.0005	Normal.
II-348	48-78	0	" "	.001	Died—4 days after injection.
II-349	28-56	0	" "	.01	Died—3 days after injection.
II-350	26-60	0	" "	.005	Died—3 days after injection.
III-361	56-80	50 days	" "	.02	Died—5 days after injection.
III-362	62-94	" "	" "	.01	Local tetanus. Recovery.
III-363	54-86	" "	" "	.005	Local tetanus. Recovery.
III-364	50-74	" "	" "	.001	Slight local tetanus. Recovery.
III-365	60-96	" "	" "	.0005	Normal.
IV-371	54-82	" "	" "	.02	Died—3 days after injection.
IV-372	48-69	" "	" "	.01	Local tetanus. Died 11 days after injection.
IV-373	52-84	" "	" "	.008	Local tetanus. Recovery.
IV-374	62-88	" "	" "	.001	Very slight local tetanus. Recovery.
IV-375	56-86	" "	" "	.0008	Normal.
V-366	64-94	0	" "	.02	Died—3 days after injection.
V-367	62-89	0	" "	.01	Died—4 days after injection.
V-368	50-77	0	" "	.005	Died—3 days after injection.
V-369	70-98	0	" "	.001	Severe local tetanus. Destroyed on 25th day.
V-370	68-96	0	" "	.0008	Severe local tetanus. Destroyed on 25th day.

4499

Radiological Study of Motility of Gastro-Intestinal Tract of Rachitic Rats.

L. J. MENVILLE, S. N. BLACKBERG AND J. N. ANÉ.
(Introduced by J. H. Musser.)

From the Department of Medicine and Department of Pharmacology, Tulane University.

Although rickets is a disease primarily affecting the skeletal parts it nevertheless produces abnormal changes in the entire body. It is usually associated with muscular weakness and relaxation and since one of the early symptoms of rickets is constipation, the following experiments were planned to compare radiologically the motility of the gastro-intestinal tract of rachitic rats with that of normals.

In these experiments 40 rats were used, 20 rachitic and 20 normal. Many of this number had to be discarded during the work for various reasons. The animals were from a uniform strain of Albino rats raised by one of us (S.N.B.) from Wistar Institute Stock. The litters were equally divided among the normal and rachitic groups. The normal groups received adequate diet. The rachitic groups were fed a rachitogenic diet, (McCollum 3143) until definite rachitic bone changes were visible by means of the x-rays. These animals were kept in individual cages in the x-ray laboratory for 3 days prior to receiving the bariumized meal, in order to let them become accustomed to the noises of the x-ray apparatus so that their motility would not be disturbed. It was shown that psychic influences such as fear and anger markedly retarded the motility of the gastro-intestinal tract. This was clearly demonstrated by the prolonged emptying time of the rats which were tied to wooden racks so as to make roentgenograms. After the rats were fasted for 48 hours and water withheld 24 hours they were given a barium meal composed of 10 gm. of barium sulphate and 10 cc. of buttermilk and were allowed to eat for 20 minutes, when they were fluoroscoped immediately in loose cotton sacks to ascertain whether their stomachs were full.

TABLE I.

Average	Normals	Rachitic
Weight of rats	210 gm.	115 gm.
Weight of food	5.8 gm.	10.5 gm.
Stomach emptying time	6 hrs. 14 min.	8 hrs. 5 min.
Appearance in cecum	3 hrs. 37 min.	2 hrs. 28 min.
Small intestine emptying time	10 hrs. 26 min.	12 hrs. 2 min.
From stomach emp. time to small intestines emp. time	4 hrs. 12 min.	4 hrs.
Colon emptying time	65 hrs.	88 hrs.

As shown in Table I the average emptying time of the stomachs of the rachitic group was somewhat longer than the average of the normal group. The stomachs of the normal rats were all of the fish-hook type and hypotonic in tone, while the rachitic rats' stomachs were also of the fish-hook type but inclined to the atonic tone. By observing the early appearance of the food column in the cecum of every rachitic rat, we believe that this demonstrates an apparent hypermotility of the small intestine in this group. The colons of the rachitic group, on the other hand showed a hypomotility as evidenced by the slow emptying of their colon as compared to the normal group. It was also observed that the colon of the rachitic group was of the atonic type, often markedly distended and lowly situated.

4500

Further Observations on Experimentally Produced Arteriovenous Aneurysm.*

I. M. GAGE AND GEORGE HERRMANN.

From the Department of Surgery and the Department of Medicine, Tulane University School of Medicine.

Arteriovenous aneurysm studies which we have been pursuing have yielded findings, difficult of immediate interpretation. We found[1] the cardiac shadow uniformly enlarged on immediate postoperative fluoroscopic examination and confirmed this with roentgenograms. These dogs had been completely anesthetized with large doses of sodium barbital, and apparently as a result the postoperative pulse rates were slower. Later we found the heart size actually decreased immediately after opening of an A-V fistula. These latter dogs had been anesthetized with ether, and their heart rates were high. Ten dogs submitted to general anesthesia for abdominal operations for the production of intestinal adhesions, with gauze and iodine, were studied before and after the induction of anesthesia and operation. There was usually a decrease in the cardiac size, especially in the region of the right ventricle. The decrease was related to the height of the rise in the heart rate. In a few of these dogs in which the heart rate remained low there was little or no decrease in heart size.

In order to study the mechanism of the thrill and the effects of the lesions, 3 types of arteriovenous aneurysms have been produced. The original type I, commonly made and observed clinically, is the side to side anastomosis with a fistula between artery and vein. The second type II consisted in suturing of the proximal end of the artery into the side of the vein, thus adding the total arterial output of the specific artery to the venous return volume of the specific vein. The third type III was produced by suturing the proximal end

---

* Aided by a grant from the David Trautman Schwartz Research Fund of The Tulane University.

[1] Gage, I. M., and Herrmann, George, PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 765, 767.

of the artery to the proximal end of the vein, ligating the distal segment of the vein as well as that of the artery.

These types of A-V aneurysms were made in the accessible peripheral arteries, in the carotid arteries and jugular veins, femoral arteries and veins. The side to side anastomoses were also made between the aorta and the inferior vena cava. The thrill was most uniformly prominent and propagated most widely in the side to side anastomoses in the carotid-jugular fistulae and usually also fairly widely in the femoral fistulae. One femoral side to side anastomosis presented no thrill, and in this instance the affected leg became gangrenous. In 3 side to side unions between the aorta and inferior vena cava, a thrill was never elicited immediately after the anastomosis was completed but developed later.

A rapid oscillation of the vein segments was studied in the various types of A-V fistulae by placing upon the vessel segment a minute cone of oiled cotton drawn out at its tip to fine fibrils to act as an indicator. The fine vibrations were detectable along both arterial and venous segments. In the first type in both systole and diastole, in the other 2 types only in systole. Clamping the proximal vein caused the disappearance of the oscillatory movement in the distal vein, while clamping the distal vein decreased slightly, but did not remove the vibratory movement of the vessel wall.

In the end to side (type II) and in the end to end (type III) anastomoses, the thrill and bruit were for the most part confined to systole with very slight if any continuation into diastole. In some of the type III unions it was not detectable at all. In the type III of end to end anastomoses between the carotid and jugular, the collapse of the latter venous segment was noted during inspiration. The changes in the heart shadow, immediately and later, were not extensive enough to be considered definite and not at all comparable to those described for the other types.

Complete pericardiectomy preceding the production of arteriovenous aneurysms of the various types has thus far not presented any evident changes in the clinical manifestation of experimental arteriovenous aneurysms.

4501

In Vitro Cultivation of a New Spirochete From Human Source.

JOHN A. LANFORD. (Introduced by C. W. Duval.)

From the Department of Pathology, Tulane University School of Medicine.

A spirochaetal organism in pure culture has been recovered from the pleural effusion of a human case of unresolved lobar pneumonia due to type IV pneumococcus.

The organism morphologically resembles very closely the *Spirochete bronchialis* of Castellani[1] which has been demonstrated in human pulmonary lesions; however, none of the workers have successfully cultivated it and in practically all of the reported instances of this infection the spirochaete was found in the sputum. Only in cases reported by Mason[2] and Lancereaux[3] were spirochetes found in the pleural effusion and not in the pulmonary secretion. Efforts to cultivate the organism and to induce lesions in lower animals were unsuccessful.

Morphology: This organism which from the classification of Noguchi might be called a spironema is variable in its morphology, even in material fresh from the human body. The length varies from 5 to 15 microns and the width from 0.2 to 0.4 micron. The spirals are not as acute as in *Treponema pallida* and there is marked irregularity in stained preparations characterized by the presence of deeply stained granules and areas faintly stained. This irregularity may be due to 2 things: a condensation of the chromatin with the appearance of small granules or to a thinning out of the cytoplasm preparatory to transverse division. The curves vary from 2 to 6 in fresh preparations and in cultures as many as 15 or more have been noted in a single spiral. No undulating membrane has been demonstrated in fresh or stained preparation. Whereas each extremity tapers to a point, which also varies in its acuteness, no flagella have been demonstrated. These spiral organisms are very motile, moving forward and turning on the long axis. There is an undulating flexion of the body together with a corkscrew like movement taking place at the same time. In old and dying preparations the curves are less acute and the motility is very sluggish and at times absent. They divide by transverse fission.

The organisms are stained best by the Romanowsky stains which bring out quite strikingly the chromatin granules, but most simple stains easily demonstrate them. They are negative to Gram's and non-acid fast.

---

[1] Castellani, *Lancet*, 1906, i, 1384; *Brit. Med. J.*, 1909, ii, 782.

[2] Mason, *Johns Hopkins Hosp. Bull.*, 1920, xxxi, 435.

[3] Lancereaux, *Presse med.*, 1909, xxvii, 556.

Fantham,[4] studying the *Spirochete bronchialis,* describes a "granule phase," apparently a progressive concentration of the cytoplasm, forming chromatin bars or granules, and this concentration ultimately forms a series of coccoid bodies in the peripheral sheath of the organism. The sheath ruptures and sets free this coccoid body which elongates and small spirochetes emerge from them. I have not yet been able to demonstrate this phase in this particular organism.

Cultivation: The organism is easily cultivated under aerobic conditions but when freshly isolated from the human case it grew more abundantly under partial anerobiasis. The first isolations were on blood agar and dextrose bouillon containing blood. Twenty-four hours after planting, the bouillon was cloudy and rich in actively motile spiral organisms. After the culture became older the organisms became longer. After several generations the organism becomes viable on plain agar but loses most of its motility. On solid media they grow as very minute colonies even smaller than the colonies of the influenza bacillus. They have no demonstrable hemolytic action on blood agar plates but in blood dextrose bouillon they cause a very slight splitting of the blood cells. When planted on serum sugar waters there is no gas formed in any of the cultures and no change demonstrated by Andrade indicator. However, using phenol red as an indicator, lactose, sacchrose, mannite, maltose and dextrine serum waters showed slightly more alkaline. Xylose and dextrose are not changed.

Animal Experiments: Three guinea pigs were inoculated with fresh material from the human case; one intradermally, one in the groin and one intraperitoneally without any effect other than the formation of a local inflammatory mass. This mass persisted for a number of days and disappeared without producing any apparent harm. Two rabbits were injected intratracheally and into the testicle with 1 cc. of the effusion fluid from the human case. These animals manifested no evidences of infection and after the first inflammatory reaction in the testicle lasting 6 to 7 days there was no demonstrable effect from the injections. Four mice were injected, 2 subcutaneously and 2 intraperitoneally with ½ cc. of the fresh effusion without any apparent effect on the mice.

A study of the sera of one guinea pig and 2 rabbits, 6 weeks after inoculation gave interesting results. The guinea pig showed no immune bodies, but the sera of both rabbits gave positive agglutination tests against the organism in dilution of 1-120 and positive complement fixation reactions. The serum of the patient showed positive agglutination in 1-120 dilution and a strongly positive complement fixation reaction. Sera from healthy human beings and also of patients giving positive Wasserman reactions showed no specific immune bodies.

---

[4] Fantham, *Ann. Trop. Med. and Parasitol.*, 1915, ix, 391.

Neo-salvarsan, intravenously, cured the patient.

This organism resembles the description of the *Spirochete bronchialis* of Castellani but differs in the absence of a demonstrable granule phase and the ease with which it is cultivated on ordinary laboratory media. It differs, too, in that all the reported cases of the *Spirochete bronchialis* have been in individuals with pulmonary lesions opening into the bronchi. It differs very markedly from all of the other known varieties of spirochetes and apparently represents a new variety which is pathogenic for man.

Illinois Branch.

Medical and Dental Arts Building, Chicago, May 15, 1929.

4502

Influence of Various Chemical Agents on the Absorption of Histamine From the Intestine.

LAMBERT F. MAMMOSER AND T. E. BOYD.

From the Department of Physiology and Pharmacology, Loyola University School of Medicine, Chicago, Illinois.

Histamine has been shown to be present in the intestinal contents of man and of some other mammals. In view of its powerful pharmacological effects, the question of its absorption has received considerable attention. It can be absorbed from the intestine of the cat in sufficient amounts to produce a marked lowering of blood pressure.[1] Under similar conditions, however, the blood pressure of the dog is not affected.[2] In man as much as 225 mg. has been taken by mouth with no noticeable effect except a moderate stimulation of gastric secretion.[3] It has not thus far been shown that any pathological condition materially affects the behavior of the intestine toward histamine. Previous workers have studied the effects of temporary interruption of the blood supply,[1] of intestinal obstruction, and of distention,[4] with negative or inconclusive results.

Spadolini[5] treated the mucosa of the dog's intestine with diluted chloroform, and observed symptoms which he attributed to an increased absorption of the products of bacterial decomposition of proteins. There was, however, no direct evidence of such a change in permeability toward any specific substance. We have studied the effect of chloroform, and of a number of other agents, on the absorption of histamine.

---

[1] Meakins, J., and Harington, C. R., *J. Pharm. Exp. Ther.*, 1923, xx, 45.

[2] Koessler, K. K., and Hanke, M. T., *J. Biol. Chem.*, 1924, lix, 889.

[3] Ivy, A. C., and Javois, A. J., *Am. J. Physiol.*, 1925, lxxi, 604.

[4] Wangensteen, O. H., and Loucks, M., *Arch. Surg.*, 1928, xvi, 1089.

[5] Spadolini, I., *Arch. fisiol.*, 1924, xxii, 435.

In small dogs, under barbital anesthesia, a rubber catheter was passed down into the duodenum from the stomach. A ligature around the pylorus fixed the catheter in place and prevented regurgitation. Blood pressure, respiration, and pancreatic secretion were recorded.

In control experiments, 5 mg. of histamine dichloride per kilo body weight was introduced into the duodenum. No change in blood pressure was ever produced. In another series of animals, we first introduced 5 to 10 cc. of chloroform, diluted with 1 to 2 volumes of paraffin oil. The chloroform usually caused a transient disturbance in the blood pressure. When recovery had occurred, and a steady level had been maintained for some time the histamine was administered. There was a prompt and prolonged fall of pressure, usually with a gradual recovery, the whole effect lasting 40 minutes to an hour or more.

Similar results were obtained when the following agents were used:

Hydrochloric acid, 0.4%, 20 to 40 cc. carbon tetrachloride, undiluted, 15 to 25 cc. Ethyl alcohol, 15 to 30% concentration, 20 to 30 cc. When alcohol was used, we substituted ether for barbital as the anesthetic.

Dog's gastric juice, of free acidity equivalent to 0.4% HCl, was also used, in amounts of 30 cc. The subsequent introduction of histamine caused only a slight fall of blood pressure, the gastric juice appearing to be less effective than simple water solution of HCl.

Sodium fluoride, in 1% solution, gave negative results. In stronger concentrations the fluoride itself produced a gradual fall of blood pressure, which was only slightly accelerated by the administration of histamine.

Some experiments were also carried out on the colon. When histamine was introduced alone, there was no effect on blood pressure. When it was preceded by alcohol or 0.4 HCl, it produced a marked fall. The other substances were not tried.

The respiratory effects were variable, and there was never a stimulation of pancreatic secretion.

Koessler and Hanke[2] found that histamine disappears from the normal dog's intestines at a rate which would be sufficient, if all passed unchanged into the circulation, to produce demonstrable effects. They conclude that it is "rendered pharmacologically inert in its passage through the intestinal wall." It seems possible that the influence of chloroform, alcohol or HCl may be to interfere with this detoxifying process, rather than directly to facilitate absorption.

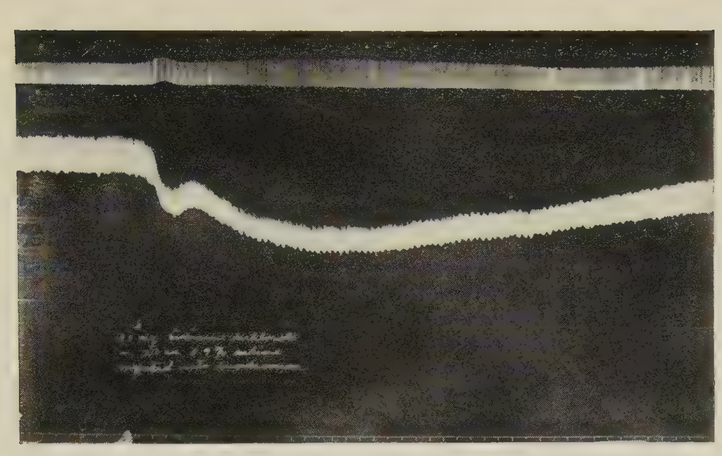


FIG. 1.

9 kilo dog, barbital anesthesia. Showing the effect of 50 mg. histamine in the duodenum. 10 cc. of chloroform in 10 cc. paraffin oil had been introduced 48 minutes previously. Time tracing in minutes. Respiration (above) and carotid blood pressure (below).

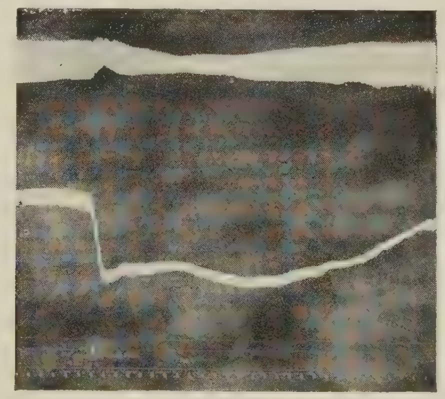


FIG. 2.

5.2 kilo dog, ether anesthesia. Effect of 55 mg. histamine (ergamine acid phosphate) in colon. 20 cc. of 30 per cent alcohol had been introduced 20 minutes previously. Respiration (above) and carotid blood pressure (below). Time, minutes.

4503

Absorption of Histamine From the Intestine of the Parathyroidectomized Dog.

R. W. ALBI AND T. E. BOYD.

From the Department of Physiology and Pharmacology, Loyola University School of Medicine, Chicago, Illinois.

In 1912 Carlson[1] described pathological changes in the stomach and upper intestine in dogs which had died as a result of parathyroidectomy. Spadolini[2] has made similar observations, and holds that the condition involves an increased permeability of the intestinal mucosa. He believes that the syndrome which follows loss of the parathyroids may be due, at least in part, to increased absorption of toxic substances produced by proteolytic bacteria. Condorelli[3] has also found changes in the intestinal mucosa following parathyroidectomy, although he does not agree with Spadolini as to their significance.

There is no direct evidence, so far as we know, that loss of the parathyroids facilitates the absorption of any specific substance from the intestine. Histamine is produced there by proteolytic bacteria, and its toxic properties are well known. As shown in the accompanying paper, the mucosa may be rendered permeable to it by relatively mild chemical agents. It seemed possible that a similar change in permeability might occur after loss of the parathyroids, in view of the extensive damage to the mucosa which has been described. If so, some support would be given to the toxemia theory of Spadolini.

Experiments were carried out on 8 dogs, each being used 6 to 8 days after removal of the parathyroids and thyroids. All of the series had shown repeated attacks of tetany. Four additional animals were operated on. Two of these never developed symptoms and were discarded. Two others died under anesthesia before the experiment was completed.

Sodium barbital was the anesthetic employed. With arterial blood pressure and respiration being recorded, ergamine acid phosphate (Burroughs, Wellcome) was introduced into the duodenum, through a tube arranged as described in the accompanying paper. The dosage was 10 mg. per kilo body weight.

---

[1] Carlson, A. J., *Am. J. Physiol.*, 1912, xxx, 338.

[2] Spadolini, I., *Arch. di fisiol.*, 1927, xxv, 739.

[3] Condorelli, Luigi, *Boll. d. soc. ital. di biol. sper.*, 1927, ii, 646.

In no instance was the blood pressure affected. The results were entirely negative, as with the controls. Koessler and Hanke[4] obtained similar negative results with normal dogs.

Of the 10 animals which had been in tetany, 9 showed no gross changes in the gastro-intestinal tract. The tenth showed a few slightly hyperemic areas in the duodenal mucosa. Microscopic examination was not made. The discrepancy between these findings and those cited above may be due to (1) the fact that our animals were killed under anesthesia before the tetany had run its full course, or (2) to the diet we gave, which was of milk and white bread.

The experiments show, however, that repeated and severe attacks of tetany may occur in the absence of any demonstrable change in permeability of the intestinal mucosa, at least toward histamine. It is still possible, of course, that the absorption of other substances may be affected.

4504

Action of Insulin on the Gastric Motility of Man.

J. P. QUIGLEY. (Introduced by A. J. Carlson.)

From the Department of Physiology of the University of Chicago.

Using the triple balloon technique it has been demonstrated that:

1. Subcutaneous injection of insulin in amounts of 12-20 units usually produces in normal human subjects fasting 11-44 hours an increase in gastric motility.

2. The essential features of this response are (a) an increase in gastric tone, (b) type A contractions, (c) a very prolonged hunger period.

3. The gastric hypermotility induced by insulin is not inhibited by smoking, mild nausea, unpleasant emotions, body discomfort, sight or thought of food, extraneous disturbances, swallowing or the presence of indifferent substances or food in the mouth or in the stomach.

4. The gastric motility is inhibited by introduction of dextrose or cane sugar into the duodenum, and also by the subcutaneous injection of atropine or epinephrine.

---

[4] Koessler, K. K., and Hanke, M. T., *J. Biol. Chem.*, 1924, lix, 889.

5. "Insulin sensations," especially hunger, parallel rather closely the degree of gastric activity.

6. Under the experimental conditions described antiperistaltic movements in the stomach were not associated with sensations of nausea.

4505

Motor and Secretory Activity of the Stomach During Acute and Chronic Obstructive Jaundice in Dogs.

KATHLEEN SULLIVAN STILL. (Introduced by A. J. Carlson.)

From the Hull Physiological Laboratory of the University of Chicago.

This work was done in an attempt to segregate the possible rôle of impaired gastric functions in jaundice.

The effect of acute and chronic obstructive jaundice on the hunger contractions of the empty stomach were studied by the balloon method 18 hours after a standard meal. The effects on the secretory functions of the stomach were studied by means of Pavlov pouch dogs. In some animals 100 gm. of hamburger was the test meal, in others 1 mg. of histamine, injected subcutaneously, was the stimulus.

Acute jaundice was produced by intravenous injection of ⅛ to ½ cc. of bile per kilo of body weight. These experiments were controlled by injection of 0.9% NaCl solution, which had no effect.

Similar studies were made on chronic obstructive jaundice after appropriate control studies. Obstructive jaundice was produced by double ligation and section of the common bile duct.

Summary: 1. Intravenous injection of ⅛ to ½ cc. of bladder bile per kilo of body weight in dogs causes an immediate but temporary inhibition of gastric motility (hunger), and greatly diminishes the gastric secretion following a test meal. The inhibitory effect upon gastric secretion is more prolonged than upon gastric motility.

2. Chronic obstructive jaundice is followed by a decrease of the motor activity of the empty stomach, a decreased rate of gastric juice secretion, and an increased acidity of the gastric juice.

4506

Fractionation of Pollen Proteins and Their Skin Reactions on Sensitive Individuals.

B. Z. RAPPAPORT AND C. A. JOHNSON.* (Introduced by Wm. H. Welker.)

From the Laboratory of Physiological Chemistry and the Department of Pathology of the College of Medicine, University of Illinois.

Dunbar[1] showed that hayfever was caused by sensitiveness to the proteins of pollen. We are attacking the problem on the following assumption: (1) That hayfever is due to sensitivity of an individual to one or more proteins of pollen. (2) That this sensitivity is due only to substances of protein nature (we are aware that evidence has been presented that sensitivity may be due to non-protein substances. (3) That the isolation of the offending protein or proteins in pure form should be the ideal therapeutic agent. Our present paper is concerned only with the separation of the proteins of giant *(Ambrosia trifida)* and common *(Ambrosia elatior)* ragweed pollen, and the results of skin reactions of the proteins obtained on sensitive individuals.

Twenty-eight gm. of pollen was exhaustively extracted in a ball-mill with the following solvents in the order indicated: Distilled water, 5% NaCl, 1% NaF, 0.25% Na_2CO_3, and 0.1% HCl. It was determined clinically that water extracted practically all of the active substances. Hence, our interest is mainly in the water soluble proteins.

To a solution of the water soluble proteins an equal volume of a saturated solution of ammonium sulphate was added. The precipitated proteins were filtered off, dissolved in water, and reprecipitated from the water solution 3 times by adding an equal volume of ammonium sulphate as indicated, finally the proteins were dissolved in water and dialyzed free from salt. The proteins which precipitated between these concentration limits with ammonium sulphate are the globulins. The filtrate from the above fraction was raised to 64% by adding saturated ammonium sulphate. The proteins which were precipitated between these limits were reprecipitated 3 times, finally dissolved in water and dialyzed the same as the previous fraction. The proteins of this fraction are no doubt a mixture of globulins and albumins. The filtrate from the above fraction was completely saturated with ammonium sulphate and the precipitated proteins of this fraction, precipitating above 64% saturation with ammonium sulphate, are predominantly of the albumin type.

---

* Lilly Research Fellow in Physiological Chemistry.

[1] Dunbar, *Berlin klin. Wchnschr.*, 1903, 24 and 26.

The subjects used for the experiment were consecutive cases giving a history of autumnal hayfever and showing a skin reaction to solutions of ragweed pollens. After the first few cases the tests were limited to 6 solutions of the water soluble proteins, the albumin, globulins and the protein precipitated at 50-64% of ammonium sulphate from the giant and common ragweeds. Each of these solutions was used in a concentration of 0.0025% nitrogen. A drop of the material placed on the volar surface of the forearm was stabbed into the superficial layers of the skin with a sharp, specially designed instrument making a 1 mm. cut not deep enough to produce bleeding. Care was taken to prevent contamination of each drop with the material preceding it. The reactions to the different protein solutions were classified in pluses in each individual by measuring the size of the wheal, the presence or absence of pseudopods, the elevation, and the extent of the erythema produced. The strongest reaction was marked a 4+, and the others graded in proportion.

The 40 cases divided themselves into 2 distinct groups, 20 being present in each group. The individuals of one group were sensitive to the protein of the common ragweed only, those of the other to the proteins of both weeds.

In the group sensitive only to the common ragweed the reaction was distinctly limited to the globulins only, a slight reaction being present in the 50-64% fraction, and no reaction in the albumin fraction.

In the group sensitive to both weeds the reaction was less limited. The skin sensitivity to the common ragweed in this group of cases again was most marked to the globulins, where invariably the reaction was classified as a 4+, and a 2+ to the albumin as well as to the 50-64% fraction of the common ragweed. The reaction to the protein of the giant ragweed given by this group was almost completely limited to the albumin fraction, very little reaction being present to any of the other proteins of the giant ragweed.

The limited amount of pollen material available for this preliminary work compelled the use of solutions of a 0.0025% N concentration limited to the technique described. This permits the conclusion that there are 2 groups of cases sensitive to the ragweed pollens. The difference may be only relative, one group being more sensitive than the other. More concentrated protein solutions and possibly the use of intradermal technique on some cases will be used to clarify this question.

4507

Efficiency and Advantages of Calcium Gluconate as a Therapeutic Agent.*

STANISLAUS A. SZUREK. (Introduced by A. B. Luckhardt.)

From the Physiological Laboratory of the University of Chicago.

Until a few years ago the progress of calcium therapy was seriously hindered by the physical and pharmacological properties of practically all calcium salts. Many are hygroscopic; practically all are nauseatingly salty-bitter to the taste; many are not well borne by the gastro-intestinal tract; and when their solutions are placed under the skin, severe, local, inflammatory reactions, leading even to actual necrosis, almost invariably follow.

Recently there has appeared 'Calcium-Sandoz," a calcium salt of gluconic acid—an oxidation product of glucose. This preparation is tasteless, soluble in water to the extent of 3% at room temperature; at higher temperatures it is 20-30% soluble. It contains 13% CaO, or 9.3% Ca. Physiologically and pharmacologically it has been found equivalent to $CaCl_2$ in equimolecular concentrations. Its medium lethal dose for mice and rabbits is much less than that of the chloride, the mannitol sulfate, and the mixture of the chloride and urea. When injected intra- or subcutaneously, or intramuscularly it causes no such severe local reactions as do the other calcium preparations just mentioned.[1] Clinically, subcutaneous and intramuscular injections are reported as relatively painless and followed by no local reactions. It has been used in the treatment of bronchial asthma, spasmophilia, pruritus, urticaria, mild tuberculosis, etc.[2, 3, 4]

These reports suggested testing calcium gluconate for preserving the lives of completely parathyroidectomized dogs, and comparing it with calcium lactate for local irritant effects. For the first purpose 9 dogs were thyroparathyroidectomized. Immediately after the operation calcium gluconate was administered according to the method of Luckhardt and Goldberg[5] except that instead of giving orally

---

* I wish to take this opportunity to thank Dr. A H. Luckhardt for the many valuable directions and suggestions given during the course of this work.

[1] Rothlin, *Schw. Med. Woch.*, 1927, 17.

[2] Kottmann, *Schw. Med. Woch.*, 1927, 18.

[3] Zimmerman, *Aertzl. Rundschau*, 1928, 7.

[4] Schaffler, *Deutsche Med. Woch.*, 1927, 42.

[5] Luckhardt and Goldberg, *J. Am. Med. Assn.*, lxxx, 79. See also Compere and Luckhardt, PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 526.

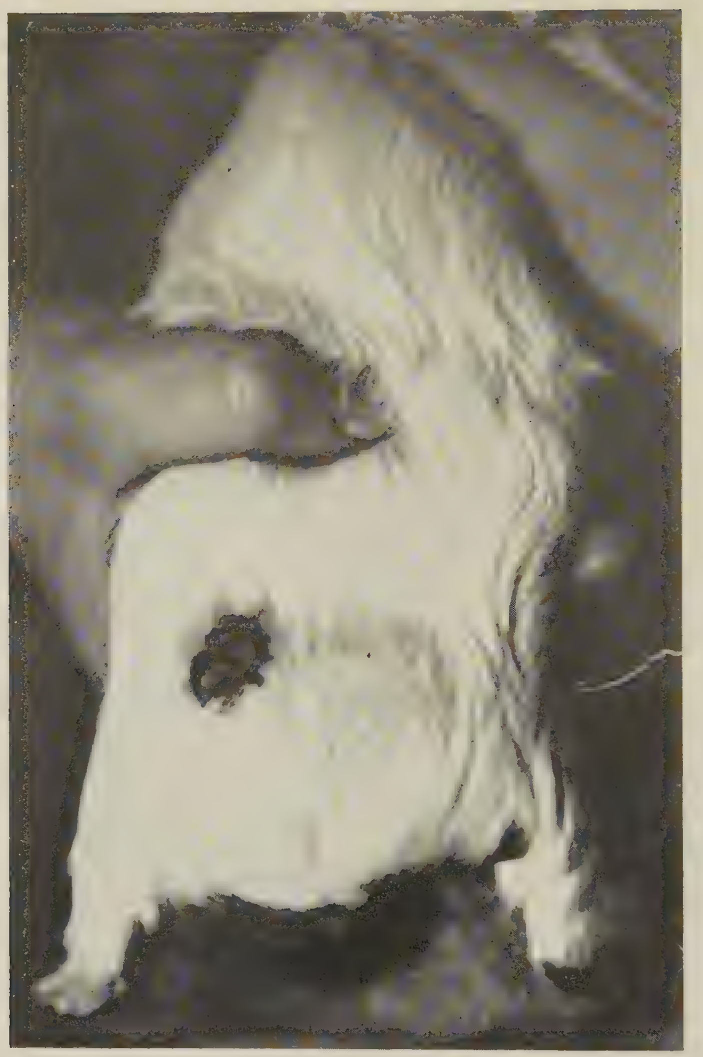


FIG 1. One cc. of calcium gluconate 10% (Sandoz) injected under the skin on the right side, and one cc. of calcium lactate 5%, on the left side 6 days before the photograph was taken. The solutions were sterile and aseptically administered.

1.5 g. per kg. per day, which is the calcium lactate dosage used by these authors, an equimolecular dose, *i. e.*, 3 g. per kg. per day, given in 3 doses, of the gluconate was found necessary to keep the animals free from tetany. Four to 6 days after the operation the animals were then tested for parathyroid deficiency by withholding the calcium gluconate until marked signs of parathyroid tetany ensued. Calcium gluconate injected intravenously (10 cc. of 10% solution) almost invariably controlled the attack within 5-15 minutes and sometimes sooner.

Of the 9 animals 2 died of an accident while the stomach tube was being passed, one lived 11, the other 4 days after the operation; a third died after 13 days from a severe attack of distemper; a fourth was found dead on the fourth day with signs of acute tetany, while being tested, by withholding the calcium, for parathyroid deficiency. Of the remaining five, 2 are still living, now in the latent stage of tetany for more than 4 months. The other 3 lived for more than 50 days after operation, *i. e.*, all had survived the acute stage for 15-30 days and were no longer regularly medicated. They succumbed to acute attacks of tetany, which killed the animals in the absence of the writer. All showed a very marked diarrhea which was only partly controlled by substituting part of the gluconate by $CaCO_3$.

In order to compare the local irritating effects of calcium gluconate with that of calcium lactate an experiment was devised which we thought somewhat better controlled than those of Rothlin[1] for a similar purpose. Eight guinea pigs were given aseptically subcutaneous injections in equal amounts of equimolecular sterile solutions of calcium gluconate and calcium lactate. The sites of injection varied in the different animals but *each* animal received *both salts,* one on each of the corresponding sides of the body. The doses injected in the various animals varied from 1 to 3 cc. Two additional pigs were similarly treated except that the salts were administered intramuscularly. One of these was found dead 2 days after the injections. In all of the remaining 9 except one, the site of the injection of the lactate showed within 24-48 hours marked induration, followed, in a few days, by severe ulceration. In all of these 8 animals the site of injection of the gluconate showed very little if any discoloration, and never any induration or inflammation. The one exception made to the 9 above was injected under the skin of the lateral walls of the thorax, and there appeared no reaction at either site of the injection, but a very large area of severe necrosis and ulceration of the whole ventral surface of the thorax and upper abdomen. Apparently the calcium lactate had gravitated promptly to the most dependent portion and produced its necrosis at that place.

Conclusions: 1. Since on the basis of the experience of many investigators of parathyroid tetany it is possible to state that the average period of survival of complete parathyroidectomy in dogs is about 3-5 days, we feel justified in concluding that calcium gluconate (Sandoz) is fully as efficient in preserving the lives of completely parathyroidectomized dogs, in equimolecular doses, as is calcium lactate. 2. In comparing the experience of investigators using calcium lactate for the control of tetany with ours, it *seems* warranted to state that calcium gluconate in the doses administered appears to have a more marked tendency to produce a severe diarrhea in parathyroidectomized dogs than does calcium lactate. This effect may offset the advantage of tastelessness as compared with bitter calcium lactate. 3. We are able to confirm the finding of Rothlin, and others that calcium gluconate is practically non-irritating when administered subcutaneously and intramuscularly, whereas calcium lactate in equivalent equimolecular doses similarly administered to the very same animal causes severe inflammation and necrosis.

4508

Cutaneous Maximous Reflex in Experimental Hypothyroidism.

M. M. KUNDE* AND MARY NEVILLE.

From the Hull Physiological Laboratory of the University of Chicago.

In the normal rabbit, light tactile stimuli applied to the dorsum, causes a marked reflex contraction of the cutaneous maximous muscle. This response is especially marked if the stimulus is applied near the tail. In the cretin this reflex completely disappears but can again be elicited if the rabbit is treated with thyroid substance. The disappearance of this reflex is a reliable sign of cretinic degeneration.

4509

Changes of Structure and Lipin Content of Central Nervous System in Ether Anesthesia.

ARTHUR WEIL. (Introduced by L. W. Ranson.)

From the Institute of Neurology, Northwestern University Medical School.

The present investigation is part of a study of the reaction of the myelin sheaths of the nerve fibers under different pathological conditions. In order to avoid the criticism brought forward against histological changes described in earlier publications,[1] material from normal animals and those which had been under ether narcosis was fixed, embedded and cut jointly, and sections of both were mounted and stained on the same slide. Changes in the arrangement of the Nissl bodies of anterior horn cells of the spinal cord could be noticed after 2 hours of narcosis. The bodies seemed to be broken into finer particles and compared with normal cells the design was more diffuse. The myelin sheaths in sections through the spinal cord and its roots (light-green-fuchsin and hematoxylin-iron stains) were distended and thinned out as by compression. The axis cylinders seemed to be compressed by the same fluid which distended the myelin sheaths.

---

* Douglas Smith Fellow.

For the study of the lipins a suggestion of Maclean[2] was transferred into practical application. The method gave workable results. It could be demonstrated that the acetone soluble substances increase considerably during narcosis, relatively more in the white matter than in the gray. This would be in accordance with the finding of lipemia in the blood during narcosis (Reicher[3]). At the same time the alcohol soluble part was diminished, most in the gray; less in the white matter and in the spinal cord. The substances which can be precipitated from the alcohol extract by acetone (raw lipins) were diminished most in the gray, less in the white, and no changes occurred in the spinal cord. Similar changes were also found in cor-

TABLE I.

Distribution of the different extracts in % of the dry substance.
N = normal animals. E = after ether narcosis from 3 to 5 hours.

		5 normal and 5 ether dogs						8 normal, 4 ether cats			
		Gray		White		Spin. Cord		Brain		Spin. Cord	
		N	E	N	E	N	E	N	E	N	E
	Water	78.9	78.8	70.4	69.4	67.4	68.0	75.2	77.9	67.8	69.7
I	Acetone extract	13.3	15.3	13.3	18.2	16.6	18.1	10.0	12.9	17.3	20.2
II	Alcohol extract	37.1	32.8	53.4	48.6	57.8	55.8	45.5	39.9	55.3	53.2
	Total extract	50.4	48.1	66.7	66.8	74.4	73.9	55.5	52.8	72.6	73.4
	Precipitate	15.9	12.9	28.2	24.2	31.3	30.5	18.0	17.4	28.0	25.7
III	Alcohol soluble	4.1	3.5	6.7	7.7	6.9	9.4	11.9	11.3	17.6	16.7
IV	Ether soluble	7.4	6.2	10.2	8.8	11.5	11.3				
V	Pyridine insoluble	2.9	2.1	4.6	3.2	5.3	3.6	6.1	6.1	10.4	9.0
VI	Pyridine soluble	1.5	1.1	6.7	4.5	7.6	6.2				
	Rest of extracts	49.6	51.9	33.3	33.2	25.4	26.1	44.5	47.2	27.4	26.6

According to H. and I. Maclean[2] the distribution of the different soluble substances is the following: I = cholesterol and neutral fats. II = rest of cholesterol and neutral fats with total lipins. The acetone precipitate of the alcohol extract contains the raw lipins. III = lecithin mainly. IV = kephalin. V = sphingomyelin mainly. VI = galactolipins mainly.

---

[1] Winterstein, H., *Die Narkose*, 2nd edition, Berlin, 1926.

[2] Maclean, Hugh and Ida, *Lecithin and allied substances*, 2nd edition, New York, 1927, pp. 12-13.

[3] Reicher, K., *Z. f. klin. Med.*, 1908, lxv, 233.

responding phosphorus analyses. The deviations from the normal values increased with the time of the anesthesia.

Experimental evidence has been established that metabolic changes are 2 to 3 times as intensive in the gray substance as they are in the white. The pathological changes in the composition of the lipins during ether narcosis run parallel to this difference in intensity between gray and white under normal conditions.

4510

Ultraviolet Transparency of the Various Layers of Human Skin.

A. BACHEM.

From the University of Illinois College of Medicine.

Frozen skin sections obtained from the sole of the foot of fresh bodies were placed over the slit of a quartz spectrograph; the surface of the skin was at right angles to the length of the slit. The spectrum of the Kromayer lamp through the visible and the whole ultraviolet was obtained on a photographic plate with the widest variation of exposure, with and without the skin sections interposed. Every spectral line showed the transmission of the various skin layers from the stratum corneum to the corium, in closest comparison for the same thickness of every layer, from 10 to 70 μ, with various sections on different plates. The absorption coefficients were calculated from these observations for every layer. These were found to vary slightly with the thickness of the section, for which observation an explanation can be found by reflection and scattering.[1] The absorption curves showed marked differences for the various layers. The corneum and the granulosum exhibited a maximum of absorption at 280 $\mu\mu$, coinciding with the maximum of the antirachitic effect as observed by Sonne.[2] This suggests that the antirachitic effect takes place in one or both of these layers. The corium and stratum germinativum do not show an increased absorption at this wave length, but exhibits a gradual increase of absorption. The production of an erythema occurs in these layers in the shadow of the upper layers. This explains the decrease in sensitivity as was observed by Hausser and Vahle at 280 $\mu\mu$.[3]

---

[1] Bachem, A., and Kunz, J., *Arch. Phys. Ther.* 1929, x, 50.

[2] Sonne, C., and Reckling, E., *Strahlenther,* 1927, xxv, 552.

[3] Hausser, K. W., und Vahle, W., *Strahlenther,* 1921, xiii, 41.

4511

Development of Elastic Fibers in Cultures of Embryonic Heart Muscle.

WILLIAM BLOOM.

From the Department of Anatomy, University of Chicago.

Because of the close association of elastic fibers with contractile tissues throughout the body, cultures of embryonic heart muscle were tested for the presence of elastic fibers. I have been unable to find a report of such an experiment. Portions of the heart of an 8 cm. guinea pig embryo and a 4 cm. rabbit embryo were cultured in homogenous embryonic extract and plasma. The cultures were made on round coverslips by Maximow's method and in Carrel flasks so that in no case was it necessary to cut them for transplantation. They were fixed after 10-17 days with one or more transplantations in Zenker-formol, embedded in colloidin and serially sectioned. They were stained with Weigert's resorcin-fuchsin method for elastic fibers and counter-stained with azo-carmine. Some of the sections were stained by Foot's method for reticulum fibrils.

Typical elastic fibers develop regularly in those cultures in which the muscle was contracting up to the time of fixation. They appear in the zone of migrating newly formed cells outside the explanted bits of heart muscle. They are thin, blue-black lines which are fairly straight and parallel to the long axis of the migrating spindle shaped cells. The fibers branch occasionally and seem to be definitely intercellular. In some places they are curled and twisted and form bizarre figures of many shapes. In a few areas the newly formed fibers interlace and form a wide or narrow meshed network. Control sections of the hearts from which these cultures were made show that the only elastic fibers in them are in the walls of the relatively small cardiac vessels.

In these experiments, elastic fibers have been produced for the first time in tissue culture. These fibers stain electively with Weigert's resorcin fuchsin method; they do not stain with Foot's method for reticulum fibers and, as they occur between the newly formed cells of the culture, there can be no question of the fact that they have developed *in vitro*.

4512

A Micro-Technique for the Determination of the Acid-Base Balance of the Blood.

N. W. SHOCK AND A. B. HASTINGS.

From the Lasker Foundation for Medical Research and the Department of Medicine, University of Chicago.

An acid-base pipette has been developed for the determination of the acid-base balance of the blood on a single specimen of 0.1 cc. Finger blood is collected under oil saturated with a mixture of 5% CO_2—95% air. The pipette consists of a lower stem, A, graduated from 0.0 to 0.1 cc. in 0.005 cc. divisions, and a bulb, B, of 2 cc. volume and 11 mm. inside diameter (Fig. 1). The volume of 2 cc. is indicated by a calibration ring on the upper stem. It is important that the bulb B be made from the test-tubes similar to those containing the standards for the pH determination.

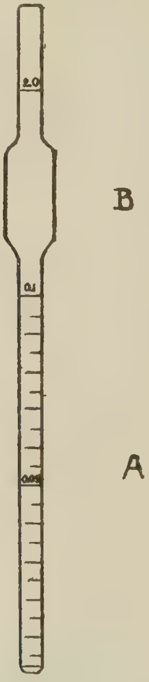


FIG. 1.

The use of the pipette is as follows: 0.1 cc. of blood is sucked into the pipette. This is followed by adjusted phenol-red saline solution up to the 2 cc. mark. The pipette is capped with a heavy rubber band and centrifuged. The volume of cells is noted, the pH is determined according to the method of Hastings and Sendroy[1] by comparison with standard tubes in a comparator block; the CO_2 content of the solution is determined by transferring the entire 2 cc. into the Van Slyke manometric blood gas apparatus and determining its CO_2 according to the method of Van Slyke and Neill.[2]

A blank CO_2 determination is made on the reagents used. The data thus obtained, namely, pH, CO_2 content, and per cent cells, are sufficient to characterize the acid-base balance of the blood. A comparison of the results obtained by this method with those obtained by the standard macro-methods have shown that the percentage cells and pH are determined with the same accuracy in both methods. An error of 2% may be expected in CO_2 determinations by this method as compared with 0.5% by the method using 1 cc. of blood. The method is proving useful in the study of acid-base balance in animals where only small amounts of blood are available and in studies on humans where frequent vena punctures are inadvisable.

---

[1] Hastings, A. B., and Sendroy, J., *J. Biol. Chem.*, 1924, lxi, 695.

[2] Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, lxi, 523.

Minnesota Branch.

University of Minnesota, May 29, 1929.

4513

Basal Metabolism of a Woman Not Secreting Ovarian Hormone After Its Injection.*

J. F. MC CLENDON AND GEORGE BURR.

From the Department of Physiological Chemistry, University of Minnesota, Minneapolis, Minnesota.

In a previous communication[1] it was shown that certain single injections of ovarian hormone were not followed by any marked rise in basal metabolism 24 hours after injection. There was usually a slight rise during the day of injection and a still slighter rise during the day with no injection. This last fact might be taken as evidence that the results were due to accidental variations or variations in the secretion of the hormone. To eliminate the last objection the following determinations were made on a 20 year old woman who had apparently been castrated by mumps at the age of 6 and whose secondary sexual characters were developed only to the pre-puberty level or about to the extent of a girl of 10-12 years of age. An assay of the blood by an extraction method showing as high as a number of mouse units per cc. in normal women, showed no ovarian hormone in this woman and she had never menstruated in her life. Previous to this experiment her basal metabolic rate was 30-34 calories per square meter per hour. The ovarian hormone was obtained from Parke Davis and was assayed by us by the single injection of the aqueous solution in ovariectomized mice. We suppose this mouse unit to be about equal to the Allen Doisy rat unit but do not doubt that there may be a variation of 1000% in individual animals.

---

* This research was aided by grants from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association and from the American Association for the Advancement of Science.

[1] McClendon, Burr and Conklin, Proc. Soc. Exp. Biol. and Med., 1928, xxvi, 265.

It may be seen from the table that the basal metabolism may rise a little on a fast-day (1st control). This rise is not increased by the pain of an injection or other psychic effect (2nd control). In this second control, distilled water was injected but the patient was led to believe that it was ovarian hormone and it was just as painful as the ovarian hormone preparation. On repeated injection of ovarian hormone during the day the basal metabolic rate rose 11.3% in the first experiment and 18% in the second experiment. The rise in metabolism on the control days is not constant and the rise after injection of hormone is not proportional to the amount injected. The hormone preparation was not pure and therefore it is not known whether the hormone or an impurity caused the rise. Claire Conklin has shown a rise in basal metabolism during the intermenstrual period of normal women of more than 2.5 times the probable error, and Wildebush and McClendon have shown a rise in the ovarian hormone in the blood during this period. This indicates that the rise in basal metabolism is due to the hormone or to the growth of the endometrium.

TABLE I.

Day	Hour	Cal. per sq. m. per hr.	Ovarian hormone injected (mouse units)	Percentage increase in b.m.r.
1	0	33.7	0	
	8	34.9	0	3.5 (1st control)
2	0	33.4	0	
3	0	31.8	0	
	0.5		250	
	2.5		350	
	6		400	
	7		500	
	8	35.4	0	11.3
4	0	32.9	0	
9	0	31.8	0	
	0.5		H_2O	
	5		H_2O	
	9	32.7	0	2.8 (2nd control)
10	0	32.8	0	
	0.5		500	
	5		500	
	9	38.7	0	18.0

4514

Variations in Basal Metabolism and Their Relation to Skin Temperature.*

J. F. MC CLENDON.

From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis, Minnesota.

The basal metabolic rates of 47 women 20-29 years old (unselected university students, born and living in a goitrous region and about 50-90% of whom have had adolescent enlargement of the thyroid) have been repeatedly determined by Claire Conklin or myself. None of them had basal metabolic rates above the Aub-Dubois standard and only 5 equal to this standard. The 17 highest averaged 35.87 calories per square meter per hour which is 3% below the Aub-Dubois standard. The remaining 30 averaged 32.15 calories per square meter per hour or 13% below the Aub-Dubois standard. This was true whether the metabolism was calculated from the O_2, CO_2 and urinary N, controlled by an alcohol check, or from the O_2 by means of the Benedict-Roth-Collins method. It was found possible to raise the metabolism of many of the 30 women in the low group to the Aub-Dubois standard in 50 hours by the subcutaneous injection of 5 mg. thyroxine (Hoffman-LaRoche) or ingestion of 25 gm. desiccated thyroid (Eli Lilly). Amenorrhea was more frequent among those with a low B.M.R. and was relieved by thyroid in 2 cases. A short menstrual cycle, 17-26 days, was more frequent in those of lower B.M.R. The menstrual cycle was shortened in one woman by lowering her B.M.R. and lengthened by raising it.

Measurements were made of muscle tone, the psychogalvanic reflex and skin temperature in relation to metabolism. The muscle tone was determined by C. A. McKinley's apparatus. The psychogalvanic reflex was determined by immersing 2 fingers in 2 vessels of salt solution kept at constant temperature. It was found that collodion used to define the margin of the zone of electrical contact of skin and salt solution gave rise to errors but that vaseline eliminated these errors. The skin temperature was measured by F. G. Benedict's method of moving a thermocouple (backed with a heat-insulator) over the skin. The temperature of the skin of the ventral

---

* This research was aided by grants from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association, and from the American Association for the Advancement of Science.

surface, under the bed clothes was determined and then the rate of fall of skin temperature after uncovering the body and with an electric fan at constant distance blowing air of constant temperature and humidity downward on the body. In general, the areas which dropped to the lowest temperatures were areas of low sensibility to cold or areas with thick subcutaneous fat. Although a higher metabolic rate (heat production) was often associated with a slower fall in skin temperature on exposure, there were many complicating factors. The fall in skin temperature on exposure was at first most rapid but after a time regulatory processes were called into play. Heat loss was influenced by anatomical differences in individuals. One individual with a tendency to maintain a relatively high general skin temperature acquired cold feet on exposure. No pulse could be detected in the feet. One finger of another individual became cold and white on exposure. Although a psycho-galvanic reflex resulted from exposure to cold, it might result from other causes as well and some of the individuals were much more emotional than others. Since muscle tone is a fibrillation similar to a voluntary contraction, it must be associated with heat production and shivering may be regarded as a special form of tone due to a reflex whose receptors are the muscle spindles.

It seems probable that a lowered thyroid function resulting from a low iodine intake during uterine life, infancy and childhood, affects the physiology of the individual in such a manner that thyroid therapy in adult life will not make the individual normal. From the standpoint of health and efficiency, it seems better to assure a higher iodine intake in pregnancy and infancy than to wait until adult life before paying attention to the thyroid.

4515

A Method for Assay of Ovarian Hormone in Blood and Urine and Relation of Assay to Menstrual Cycle.*

F. F. WILDEBUSH and J. F. MC CLENDON.

From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis, Minnesota.

Ten cc. of blood are drawn from the Median Basilic vein into oxalate to prevent coagulation. The blood is centrifuged, and the blood plasma pipetted off. The blood corpuscles are washed with

---

* This research was aided by grants from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association, and from the American Association for the Advancement of Science.

normal saline solution twice, and the washing fluid is added to the blood plasma. Since no ovarian hormone was found in the blood corpuscles they are not included in the extraction. To the blood plasma and saline is added about 5 cc. of *N* NaOH. This is extracted 3 times with an equal volume of ether (free of peroxides). The ether extract is put into a distilling flask and the ether boiled off under reduced pressure. The residue is taken up in normal saline. For purposes of quantitative assays, no further purification is necessary, as this extract is not so irritating as to induce sloughs in mice.

Castrated white mice are used, which have been observed to be in continuous dioestrus pause, for at least 2 weeks following castration; and when used again after having been injected, at least 4 days of dioestrus are necessary.

Urine: Twenty-four hour urine specimens are obtained from normal non-pregnant women. Five cc. of chloroform is used as a preservative. The urine is made alkaline to phenolphthalein with NaOH, and then extracted 3 times with one-fourth pound of ether for each extraction. (When the urine is acidified before extraction with ether, toxic substances pass into the ether. In most of our experiments this acid-extract caused death to the mouse in 2 hours.) The ether extract of the alkalinized urine is then treated the same as the blood extract, and the residue taken up in normal saline.

In assaying this hormone, because of the large number of assays of samples to be made, we employed a single injection instead of the 3 injections of the Allen-Doisy method or the 8 injections of the Laquer method. Readings were taken twice daily for 72 hours following injection into the mice.

The following results were obtained in a normal non-pregnant young woman:

Days of Cycle	Mouse units in 20 cc. blood
2nd day menstruation	126
3rd " "	126
6th " "	24
2nd " after menses	31
7th " " "	33
13th " " "	43
18th " " "	120
21st " " "	124
24th " " "	126

	Mouse units in 24 hr.
12 days preceding menses	33
8 " " "	36
3 " " "	42
1 " " "	48

2nd day menstruation	33
3rd ” ”	12
1st day after menses	12
4th ” ” ”	15
5th ” ” ”	18
7th ” ” ”	30

In 2 cases of young adult women, who had never menstruated, and in whom sexual infantilism had been diagnosed, no hormone could be found in the circulating blood.

From our tables, it is seen that there is no sudden fall in the concentration of the hormone in the blood just preceding menstruation as reported by Frank and Goldberger and by Hirsch. Our tables do not support the assumption that menstruation is due to the sudden drop of this hormone in the blood.

Assuming an average of 7 liters of blood in the woman, we have computed at least 45,000 mouse units of hormone circulating in the blood stream, with no way of ascertaining the amount in storage in different organs. It appears to us, that to obtain clinical results in definite cases of hypofunction of the ovary, massive doses of the hormone must be given to approximate the amount found in a normally functioning woman.

4516

Reaction Between Antiseptics and Proteins and Antiseptic Activity of the Adsorbed Portion.

A. D. HIRSCHFELDER AND H. N. WRIGHT.*

From the Department of Pharmacology, University of Minnesota.

It is a well know fact that the action of antiseptics upon bacteria is greatly diminished by the presence of proteins, blood serum, tissue juices and pus, but whether this is due to a chemical combination with the protein or to adsorption of the antiseptic on the surface of the protein, is not so certain.

Mathews,[1] Heidenhain,[2] and Chapman, Greenberg and Schmidt,[3] who advocated the theory of chemical combination, dealt with denatured lyophobic protein and not with protein in the natural lyophilic state; this has given rise to serious misconceptions. Ashley Cooper[4] has advanced some evidence that proteins adsorb phenols and do not enter into true chemical combination with them. The experiments of Grollmann[5] on the binding of phenol red indicate that the dyes are adsorbed, but the values (*i. .e,* 1/N approximating 1) obtained lay too close to agreement with the mass law equation also to make fine distinctions possible. Rosenthal[6] also obtained an adsorption curve for the binding of rose bengal on a variety of proteins in acid solution.

---

* The experiments reported in this paper form the basis for a part of a Thesis presented by Harold N. Wright in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Minnesota (May, 1929).

[1] Mathews, A. P., *Am. J. Physiol.*, 1898, i, 445.

[2] Heidenhain, M., *Arch. f. d. ges. Physiol.*, 1902, xc, 115.

[3] Chapman, L. W., Greenberg, D. M., and Schmidt, C. L. A., *J. Biol. Chem.*, 1927, lxxii, 707.

If the reaction with protein is one of chemical combination, it should proceed in direct proportion to the reacting masses, while if it is an adsorption, it should follow the Freundlich adsorption isotherm $(X/N = KCl/n)$ and give a logarithmic curve.

In order to study the reaction with proteins in their natural state, the protein (purified egg albumin) and dye were placed in a small wide-mouthed bottle containing, as a dialyzer, a segment of viscose sausage skin filled with water. The free crystalloid dye passes through the dialyzer until its concentration in the outside chamber is equal to that within, while any portion that is in colloidal form does not do so. We soon found that some of the antiseptic dyes like mercurochrome and acriflavine, which are generally supposed to be crystalloids, actually are partly in crystalloid and partly in colloidal form; and that physiological NaCl increases the amount of the colloidal form of these dyes, as well as converts a portion of the triphenyl methane dyes into colloidal suspensions, especially at pH 7 to 8.

We have found that egg albumin in 0.3 and 1.0% solutions at pH 7.5, adsorb crystal violet, brilliant green and malachite green, which show the following values for 1/N: crystal violet 0.83, brilliant green 0.57, and malachite green 0.25. Crystal violet follows the adsorption isotherm only in the range (less than 50 mg. per 100 cc.) in which relatively little precipitation occurs. Mercurochrome and acriflavine were themselves too colloidal to permit of the use of this method of study.

The adsorption could be followed under the ultramicroscope, the soap-bubble-like appearance of the hydrated protein giving way to the star-like reflections of the larger and less hydrated aggregates.

---

[4] Cooper, E. A., and Sanders, E., *J. Phys. Chem.*, 1927, xxxi, 1.

[5] Grollmann, A., *J. Biol. Chem.*, 1925, lxiv, 141.

[6] Rosenthal, S. M., *J. Pharmacol. and Exp. Therap.*, 1926, xxix, 521.

In studying the problem of antisepsis in the presence of protein, it is important to determine whether it is only the unadsorbed fraction of the dye which acts as an antiseptic, or whether the portion which is adsorbed on the protein also exerts this action. This was tested upon the amount of CO_2 production by yeast grown in sucrose solution. Taking our curves for antiseptic strength in water alone with the curve of antisepsis in the presence of protein, and comparing these with the adsorption curves, it is evident that not only the free crystalloid, but also the adsorbed fraction, exert antiseptic action. The adsorbed fraction, however, exerts only a part of the action that would correspond to the amount of dye present.

Thus, crystal violet in a concentration of 1/20,000 in the presence of 1% albumin should behave like a solution of 1/125,000 concentration if only the free dye is actively antiseptic. Experimentally it was found to behave like a crystal violet solution of 1/35,000 concentration.

4517

Is Schnitzer's "Interference" Between the Action of Two Chemotherapeutic Substances Due to Surface Reactions?

A. D. HIRSCHFELDER AND H. N. WRIGHT.*

From the Department of Pharmacology, University of Minnesota.

Browning and Gulbransen[1] discovered the remarkable fact that although the injection of basic fuchsin cured rats infected with trypanosomiasis and acriflavine injections had the same effect, if animals previously fed basic fuchsin by mouth were infected with fuchsin-fast trypanosomes, and later acriflavine was injected, the therapeutic action of the acriflavine was prevented and the animals died of trypanosomiasis. They gave to this action the name of "interference phenomenon." These observations were confirmed by Schnitzer[2] (both with fuchsin-fast and normal trypanosomes) who showed that it applied also to other triphenyl methane dyes, *e. g.*, methyl violet and brilliant green, and to arsenicals, *e. g.*, arsphenamine and arsacetin.

---

*The experiments reported in this paper form the basis for a part of a Thesis presented by Harold N. Wright in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Minnesota (May, 1929).

[1] Browning, C. H., and Gulbransen, R., *J. Path. and Bact.*, 1922, xxv, 395.

[2] Schnitzer, R., *et al.*, *Z. f. Immunitatsförsch. u. exper. Therap.*, 1926, xlvii, 116; xlviii, 23; xlix, 387, 393, 551; 1927, liii, 439; liv, 324.

Since these experiments seem to raise questions that are fundamental in the problem of chemotherapy, it is important to determine whether they can be explained as merely surface reactions upon the trypanosomes or whether one must have recourse to other more abstruse processes of metabolism or vital phenomena.

We have therefore attempted to determine whether a similar interference could be demonstrated upon the growth and CO_2 production by yeast in the test-tube. Our curves of CO_2 production by ordinary baker's yeast grown in a sucrose solution show that yeasts which have been definitely stained by methyl violet or by brilliant green in the test-tube, in solutions too weak to affect the CO_2 production, are less sensitive to acriflavine than are normal yeast cells. Vice versa, yeast cells stained with acriflavine in concentration too low to affect CO_2 production, are rendered less sensitive to methyl violet and brilliant green. This effect is not noticeable with basic fuchsin.

These experiments represent a complete parallelism *in vitro* to Browning's and Schnitzer's "interference phenomenon" *in vivo*, and render it probable that the latter can be explained as a simple surface reaction.

4518

Ultramicroscopic Studies Upon Colloidal State of Antiseptics and Arsenicals in Relation to Their Actions.*

H. N. WRIGHT AND A. D. HIRSCHFELDER.

From the Department of Pharmacology, University of Minnesota.

As Voegtlin[1] and others have shown that the action and toxicity of arsphenamine is closely related to their viscosity and colloidality, and as the action of antiseptics is greatly diminished by the presence of protein and of lipoids (Hirschfelder and Decherd[2]) we have studied the appearance of solutions of a number of these substances under the Szigmondy slit ultramicroscope. Solutions of arsphenamine and neoarsphenamine show the soap-bubble-like appearance of hydrated lyophillic colloids, the micellae being smallest between pH 7 and 9, and again from 11 to 13. In the presence of proteins they aggregate to form stellate, highly refractive micellae. Rivanol is fluorescent, lyophillic, soap-bubble-like and aggregates with proteins. Triphenyl methane dyes are crystalloidal, but aggregate with proteins. Quinine and the hydrocuprein series (optochin, eucupin and vuzin) are fluorescent, crystalloid and aggregate with proteins. Metaphen is crystalloid and produces little change in the proteins. Mercurochrome and acriflavine show fluorescence, but no visible particles; the micellae formed by mercurochrome and protein appear to be quite small; with acriflavine the protein aggregates are somewhat larger.

---

* The experiments reported in this paper form the basis for a part of a Thesis presented by Harold N. Wright in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Minnesota (May, 1929).

We take pleasure in expressing our thanks to Dr. R. A. Gortner for his cordial cooperation and for the use of the Szigmondy ultramicroscope.

[1] Voegtlin, C., *U. S. Pub. Health Rep.*, 1924, xxxix, Part I, 179.

[2] Hirschfelder, A. D., and Decherd, G. M., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 824.

These colloidal phenomena are probably associated with the toxicity and pharmacological action of the drugs, especially in intravenous injection.

4519

Determination of Oxyhemoglobin by Means of the Duboscq Colorimeter.

B. A. FLESCHE AND J. F. MC CLENDON.

From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis, Minnesota.

In the determination of oxyhemoglobin by the Dare method the glass standard is never of the same color as oxyhemoglobin and therefore a color match is impossible. A better color match has been obtained by determining acid hematin as in the Sahli and Newcomer methods. The color match is not perfect, however, and an hour wait is required for all of the hemoglobin to be changed to acid hematin. If monochromatic light is used the difficulty of matching colors is eliminated but for practical reasons the wavelength should be one in which the blood pigment shows considerable absorption. The Wratten color filter 74, epsilon, shows a high transmission at 540 mμ and the center of one of the absorption bands of oxyhemoglobin is at 541.7 mμ. Furthermore, the transmission of this Wratten filter is in so narrow a band as to appear monochromatic to the eye. Hence the Newcomer glass standard may be used for determining oxyhemoglobin if this Wratten filter is placed in the eyepiece of the colorimeter. A slight change in the spectrum of the Newcomer glass as has been made by Bausch and

Lomb, does not prevent a color match being made. Diluting the blood 1-502 with distilled water resulted in complete laking in 15 minutes so that only this amount of time was required before making the reading. After a number of hours the formation of methemoglobin changed the reading, so it is necessary to take the reading between 15 minutes and 3 hours after diluting the blood.

If blood is to be collected during the night and the colorimeter readings made next day, the Newcomer method is preferable, but if the readings are to be made immediately (as during a physiological experiment) the present method is preferable. By substituting the Wratten filter for the B. and L. blue glass and using a different conversion table, the apparatus made for the Newcomer method may be used in this method. We prepared such a conversion table but since Bausch and Lomb have changed their Newcomer glass standards about four times, we do not reproduce it.

4520

Action of Splanchnic Nerves on Glomerular Blood Flow and Mechanism of Reflex Aneuria in Frog's Kidney.*

RAYMOND N. BIETER. (Introduced by A. D. Hirschfelder.)

From the Department of Pharmacology, University of Minnesota.

The present opinion of the splanchnic nerves upon the function of the kidney is that these sympathetic nerve fibers exert a primarily vascular effect and thus modify the size of the organ and its output of urine.

Experiments upon the living kidneys of *Rana pipiens* and *Rana catasbiana* in which the glomeruli were observed under the microscope by "Richards' Method" have shown the following findings:

1. Section of the splanchnics increases the number of glomeruli showing active blood flow.
2. Electrical stimulation of the peripheral end of the cut splanchnic trunks decreases the number of glomeruli showing active blood flow.
3. Electrical stimulation of the central ends of cut sensory nerves, the application of irritants to the ureter externally and in-

---

* The experiments reported in this paper form the basis for a part of a Thesis presented by Raymond N. Bieter in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Minnesota (May, 1929.)

ternally and to the inside of the tubules, and the entrance of edema fluid into the general circulation, lead to decrease of glomerular blood flow.

4. The effects of the measures described in (3) are lost by section of the splanchnics or anesthesia of the part stimulated.

These results tend to support the current conception of the vascular effects of the splanchnic nerves upon the kidney, and to furnish an explanation for the genesis of reflex aneuria.

4521

Influence of Diet on the Phospholipid Fatty Acids of Growing Rats.

ROBERT GORDON SINCLAIR. (Introduced by W. R. Bloor.)

From the Department of Biochemistry and Pharmacology, The University of Rochester School of Medicine and Dentistry, Rochester, New York.

Evidence has been presented[1] to show that the type of diet has a characteristic influence on the degree of unsaturation of the phospholipid fatty acids of various tissues of the cat. This influence of food fat may be divided into 2 categories: (1) in the intestinal mucosa and in the liver the phospholipid is involved in the absorption and assimilation of fat and undergoes an immediate change in composition during the absorption of a characteristic fat; (2) in all tissues studied (mucosa, liver, smooth and skeletal muscle) the degree of unsaturation of the phospholipid fatty acids is characteristic of the type of diet fed over a considerable period of time. This latter influence is probably the result of the utilization of the fatty acids of the food for the repair of the phospholipid broken down by the continuous wear and tear of cellular protoplasm.

The data presented in the accompanying table show that the type of diet has a distinct and uniform influence on the constitution of the phospholipid synthesized by young growing rats. Likewise the values given for the iodine numbers of the fatty acids of the neutral fat show the unmistakable influence of diet, thus confirming the findings of Shioji[2] and Anderson and Mendel.[3]

Two facts are outstanding. It is evident that the neutral fat of the young control rats is similar in composition to that synthesized

---

[1] Sinclair, R. G., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 436; *J. Biol. Chem.*, 1929, lxxxii, 117.

[2] Shioji, E., *J. Biochem.*, Japan, 1924, iv, 43.

[3] Anderson, W. E., and Mendel, L. B., *J. Biol. Chem.*, 1928, lxxvi, 729.

TABLE I.

Influence of Diet on Lipids of the Rat.

Group*	Growth period	Live weight			Phospholipid fatty acids			Neutral fat			Unsaponifiable
					Weight in		Iodine No.	Weight in moist tissue	Fatty acids		Weight in moist tissue
		Initial	Final	Increase	Moist tissue	Dry tissue			%	Iodine No.	
	days	gm.	gm.	%	%	%		%			%
Controls		32.5			1.17	6.78	121	6.80	93	63	0.323
		34.5			1.19	6.76	124	7.26	95	63	0.334
		21.5†			1.26	6.98	120	0.79	94	76	0.488
A	31	31.5	91.0	189	1.03	4.35	104	6.46	96	64	0.374
	31	30.0	98.0	227	1.00	4.34	103	6.08	96	63	0.325
	35	26.0	80.5	210	0.97	4.26	102	11.49	96	62	0.317
B	23	32.5	97.5	200	1.01	4.88	124	10.20	98	83	0.452
	23	27.0	87.0	222	1.04	4.92	124	9.70	97	82	0.374
	35	25.5	83.0	226	0.98	4.62	125	11.43	96	81	0.275

* Diet for Group A consisted of: 40% fat-free casein, 56% cane sugar, 4% Osborne-Mendel salt mixture.

Diet for Group B consisted of: 40% fat-free casein, 26% cane sugar, 4% Osborne-Mendel salt mixture and 30% olive oil.

Each rat received daily 0.5 gm. Northwestern dry yeast (extracted with ether) and 0.4 mg. of Oscodal.

† This rat fasted for one day before death.

by the rats raised on a totally fat-free diet, while the phospholipid fatty acids of the latter are distinctly more saturated than those of the controls. This suggests that in the young controls (which had only recently been weaned) the neutral fat was synthesized from carbohydrate, but that the food contained a sufficient amount of unsaturated fatty acids to produce a highly unsaturated phospholipid.

The fact that the addition of olive oil to the fat-free diet causes the synthesis of a highly unsaturated phospholipid is in agreement with the observation of Eckstein[4] that oleic acid stimulates the production of arachidonic and linoleic acids.

4522

Effect of Inanition on the Phospholipid Fatty Acids of the Rat.

ROBERT GORDON SINCLAIR. (Introduced by W. R. Bloor.)

From the Department of Biochemistry and Pharmacology, The University of Rochester School of Medicine and Dentistry, Rochester, New York.

Terroine and Belin[1] have asserted that the lipid component of the *élément constant* of whole animals and individual tissues represents the phospholipids of normal animals, and that the degree of unsaturation of the fatty acids of the *élément constant* (*i. e.,* the phospholipid) is an invariable characteristic, quite independent of the type of diet. The latter assertion is quite at variance with the observations of the author.[2]

The *élément constant* of Terroine represents the residual substance of animals which have died of inanition. On the other hand, the data obtained by the author apply solely to the acetone-insoluble lipids of normal, well-fed animals. This distinct difference suggests the possibility that normal animals contain reserve phospholipid which alone is influenced in its composition by the type of diet and which disappears on fasting.

The data in the accompanying table show that in fasting rats the decrease in phospholipid fatty acids is roughly proportional to the decrease in the weight of the animal, or, in other words, the percentage of phospholipid in the whole rat remains relatively constant. Furthermore there is no change in the composition of the phospholipid during fasting, the iodine number of the fatty acids remaining constant.

These facts indicate that the phospholipid of normal animals does not consist of a stable portion, invariable in amount and in composition, and a labile reserve portion. On the contrary these data strengthen the opinion that the phospholipids are vital constituents of the protoplasm, that they are subject to wear and tear, and that the fatty acids of the food are utilized in the process of repair.

---

[4] Eckstein, H. C., *J. Biol. Chem.*, 1929, lxxxi, 613.

[1] Terroine, E. F., and Belin, P., *Bull. Soc. chim. biol.*, 1927, ix, 12.

[2] Sinclair, R. G., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 436; *J. Biol. Chem.*, 1929, lxxxii, 117.

TABLE I.

Effect of Fasting on Lipids of the Rat.

Experimental conditions	Live Weight			Phospholipid fatty acids						Neutral fat			Unsaponifiable
				Weight					Iodine No.	Weight in moist tissue	Fatty acids		Weight in moist tissue
	Initial	Final.	Decrease.	Absolute	Estimated decrease*		In moist tissue	In dry tissue			%	I.N.	
	gm.	gm.	%	gm.	gm.	%	%	%		%			%
Controls	102.0			1.125			1.10	5.49	127	5.92	95	67	0.312
	108.5			1.165			1.07	5.30	126	5.93	95	67	0.288
Fasted: 3 days	103.0	85.5	17	0.900	0.21	19	1.05	4.57	127	8.58	94	67	0.282
	103.0	76.5	26	0.830	0.28	25	1.08	4.82	130	1.70	96	69	0.402
5 days	103.5	83.0	20	0.795	0.32	29	0.96	4.30	125	6.57	94	69	0.386
	106.5	83.0	22	0.830	0.32	28	1.00	4.32	127	8.71	95	67	0.518

* Estimated decrease in phospholipid fatty acids = (initial wt. of rat × 1.08) — absolute wt. of phospholipid fatty acids.

4523

Toxic and Antagonistic Properties of Na, K, Mg and Ca Ions on Duration of Life of Cambarus clarkii.

O. M. HELFF.

From the Zoological Laboratory, University of Iowa.

The toxicity of pure solutions of metallic ions has been determined in the past for many marine species. Furthermore, the antagonistic properties of small amounts of certain ions to alleviate or even remove entirely the toxic effect of another ion is well known. In a survey of the literature covering this subject, the writer became aware of the extreme paucity of work in connection with fresh-water forms. Since the fresh-water crayfish was found to lend itself to this work and to be comparable to closely allied marine forms, a species *(Cambarus clarkii)* obtainable in abundance was used in the present work.

The poisonous effects of pure solutions of NaCl, KCl and $CaCl_2$ were determined in the following manner: 0.5 M solutions were prepared with distilled water as well as a series of weaker solutions having the strength of 90%, 80%, 60%, 40%, 20% and 10% of the 0.5 M solutions. Ten battery jars were each filled with 600 cc. of the solution to be tested and a crayfish placed in each jar. A record was kept of the time of immersion of the animals and hourly inspections made thereafter to determine the duration of life. To guard against possible asphyxiation due to low oxygen tensions, the animals were placed in fresh solutions every 12 hours. This likewise removed the possible toxic effects of waste materials liberated by the crayfish. Table I gives the average result of 10 individuals for each solution:

TABLE I.

Concentration of NaCl, $CaCl_2$ and KCl solutions	Duration of Life in Hours		
	NaCl	$CaCl_2$	KCl
100 cc. 0.5 M solution + 0 cc. distilled water	20.0	17.0	2.5
90 ” ” + 10 ” ” ”	36.2	25.2	2.9
80 ” ” + 20 ” ” ”	65.9	25.3	3.0
60 ” ” + 40 ” ” ”	157.3(*7)	32.4	3.9
40 ” ” + 60 ” ” ”	160.2(*6)	43.1	4.9
20 ” ” + 80 ” ” ”	(*10)	78.8(*2)	6.5
10 ” ” + 90 ” ” ”	(*10)	95.2(*1)	17.2
0 ” ” + 100 ” ” ”	(*10)	(*10)	(*10)

* Denotes number living for 7 days when test was discontinued.

The results as shown in Table I clearly indicate the toxicity of certain metallic ions when applied in fairly high concentrations. The poisonous effect of the K ion is especially pronounced, being approximately 8 and 7 times more toxic, respectively, than equimolecular 0.5 M NaCl and $CaCl_2$ solutions.

The possible antagonistic properties of small amounts of other ions in minimizing the toxicity of K and Na ions were also investigated and results obtained as shown in Table II. The average life duration of 10 crayfish is given for each solution.

TABLE II.

Nature of the Solution	Duration of Life in hours
96 cc. 0.5 M NaCl + 4 cc. 0.5 M $MgCl_2$	22.7
” ” + 4 cc. 0.5 M KCl	21.3
” ” + 4 cc. 0.5 M $CaCl_2$	26.0
” ” + 2 cc. M $MgCl_2$ + 2 cc. 0.5 M KCl	24.0
” ” + 2 cc. 0.5 M $MgCl_2$ + 2 cc. 0.5 M $CaCl_2$	25.4
” ” + 2 cc. 0.5 M $CaCl_2$ + 2 cc. 0.5 M KCl	33.8
96 cc 0.5 M KCl + 4 cc. 0.5 M $MgCl_2$	4.1
” ” + 4 cc. 0.5 M NaCl	4.0
” ” + 4 cc. 0.5 M $CaCl_2$·	4.2
” ” + 2 cc. 0.5 M $MgCl_2$ + 2 cc. 0.5 M $CaCl_2$	4.2
” ” + 2 cc. 0.5 M $MgCl_2$ + 2 cc. 0.5 M NaCl	4.3
” ” + 2 cc. 0.5 M $CaCl_2$ + 2 cc. 0.5 M NaCl	4.3

A comparison of the results as shown in Tables I and II will make evident the apparent antagonistic properties of the various ions tested. Mg, K and Ca ions all appear to partially offset the toxic effect of the Na ion, while the combination of small amounts of Ca and K ions would appear to be especially effective. Furthermore, Na, Ca and Mg ions appear to be considerably more effective in alleviating the poisonous effect of the K ion.

Experiments are in progress testing the toxic effect of pure $MgCl_2$ solutions and the antagonistic properties of combinations of ions to pure solutions of $MgCl_2$ and $CaCl_2$. The effect of increasing amounts of antagonistic ions in pure solutions of NaCl, KCl, $MgCl_2$ and $CaCl_2$ is also being worked out.

4524

Maternally Transmitted Immunity to Bacillus Sordellii.

IVAN C. HALL.

From the Department of Bacteriology and Public Health, University of Colorado School of Medicine.

During our efforts[1] to produce antitoxic and agglutinating serums against *Bacillus sordellii (C. oedematoides),* one of our immunized rabbits accidentally became pregnant and gave birth to 10 young of which she succeeded in raising 4. When these were weaned it occurred to me to test their immunity toward the organism which had been used in the immunization of their mother.

Since details of immunization of the mother preceding and during gestation, as well as during lactation may be of some significance for the interpretation of the immunity conferred upon her young, they are here noted in tabular form.

Female rabbit No. 261 was immunized against *B. sordellii* 1302 as shown in Table I.

The following schedule is submitted in detail as an example of successful immunization against whole cultures of *B. sordellii.* We have never succeeded in immunizing rabbits against whole cultures by the intravenous route and sacrificed many in attempting to do so. Even animals as completely immunized as this rabbit was at the end, have succumbed to small doses of whole cultures administered into the vein.

It is clear that this rabbit possessed a well established antitoxic immunity before or about the time gestation started. This is shown by the fact that in spite of the relatively large doses of toxin received, she continued to gain weight and showed no alarming symptoms, only a moderate degree of localized oedema after each subcutaneous inoculation. Serum separated from the blood drawn after the first week of gestation also possessed definite protective power for guinea pigs inoculated simultaneously with filtered cultures of *B. sordellii* and *C. oedematoides.* Furthermore, repeated inoculations of this pregnant rabbit did not cause her to abort, as so often happens, and she continued to gain weight both during gestation and after parturition in spite of constantly increasing doses of first toxin, and later, whole cultures. However, she lost 6 of the young at an early age from an unknown cause. When the 4 re-

---

[1] Hall, I. C., Rymer, M. R., and Jungherr, E., to be published in the *Journal of Infectious Diseases.*

TABLE I.

Date	Wt. in gm.	Dose
Mar. 7	1500	1 cc. (1-10) toxin B* intravenously.
" 12	1500	1 " (1-9) " " "
" 15	1600	1 " (1-8) " " "
" 19	1650	1 " (1-6) " " "
" 22	1750	1 " (1-4) " " subcutaneously.
" 26	1875	1 " (1-3) " " "
" 29	1875	1 " (1-2) " " "
Apr. 2	2000	0.7 " " " "
" 5	2050	0.8 " " " "
" 10	2250	1.25 " " " "
" 13	2350	2 " " " "
" 17	2450	2 " " " "
" 20	2600	2 " " " "
" 25	2700	2 " toxin G† "
" 28	2750	3 " " " "
		Accidentally bred about this time.
May 2	2900	4 cc. toxin G subcutaneously.
" 8		Bled about 30 cc. from heart; 1 cc. serum protects guinea pigs against 4 M.L.D. of toxin.
" 9	3050	
" 12	3150	5 cc. toxin G subcutaneously.
" 15	3225	5 " " " "
" 19	3300	
" 26	3500	1 cc. 7 day whole culture subcutaneously.
" 29	3550	1 " 2 " " " "
" 30	3100	*10 young born.*
June 1	3250	1 cc. 1 day whole culture subcutaneously.
" 6	3250	1.7 " 4 " " " "
" 9	3450	1.7 " 3 " " " "
" 15	3550	2 " 3 " " " "
" 19	3700	2 " 3 " " " "
" 22	3625	2 " 3 " " " "
" 25	3750	2 " 3 " " " "
" 28	3750	2 " 3 " " " "
July 6	3750	Bled about 15 cc. from ear. Agglutination titre 1-2000; 4 remaining young weaned.
" 11	3500	2 cc. 1 day whole culture subcutaneously.
" 14	3600	5 " 2 " " " "
" 19	3550	5 " 2 " " " "
" 24	3600	5 " 2 " " " "
Aug. 1	3600	Bled about 15 cc. from ear. Agglutination titre 1-5120.

* Toxin B contained less than 1 guinea pig M.L.D. per cc.
† Toxin G contained 2 guinea pig M.L.D. per cc.

maining young were weaned at 5 weeks, the agglutination titre of the mother was about 1-5120 against all 6 strains of *B. sordellii* and *C. oedematoides.*

July 11, the young rabbits were tested for any immunity that may have been transmitted to them from the mother, either congenitally or through her milk, by injecting them subcutaneously with different sized doses of a 24 hour glucose broth culture of *B. sordellii* 1302, the same strain used in the immunization of the mother. A somewhat larger rabbit from another litter was used as a normal control; this animal received the smallest dose (0.1 cc.) similarly to one of the supposed immune litter. The results are shown in the following protocol:

TABLE II.

Rabbit No.	Weight	Dose	Results
	gm.	cc.	
300	600	0.1	No effect, gained 250 gm. in 7 days.
202	625	0.5	Marked oedema; died on 7th day.
235	700	2.5	Marked oedema; died on 3rd day.
209	735	10.0	Died during 1st day.
197*	1200	0.1	Marked oedema; died on 3rd day.

* Normal control.

Rabbit No. 300 was finally killed November 2, 1928, by a blow on the head. It then weighed 2600 gm. and was normal in every way. All of the other animals that died showed the characteristic lesions of infection by *B. sordellii, i. e.,* extensive subcutaneous oedema, with a little congestion but no emphysema.

The normal control rabbit, though much larger than the immune rabbits, showed approximately the same development of lesions and died in about the same time as the immune test rabbit (No. 235) which received 25 times as large a dose of the culture. The results clearly indicated that immunity to *B. sordellii* may be transmitted from female rabbits to their offspring, but whether by placental transmission or by lactation is, of course, impossible to state from this experiment.

4525

The Buffered Diluent for Schick Toxin.

W. E. BUNNEY and BENJAMIN WHITE.

From the Antitoxin and Vaccine Laboratory of the Massachusetts Department of Public Health.

The instability of diphtheria toxin in diluted state has always presented obstacles to the preparation of outfits for the Schick test. It has been the custom to supply a small amount of undiluted toxin (or toxin in a small amount of glycerin) in one container and the required amount of saline diluent in another container, which required the mixing of toxin and diluent by the physician at the time of performing the Schick tests. A ready-made dilution of toxin which would retain its toxicity under the varying conditions to which Schick outfits are usually subjected would make for lower cost of manufacture, greater convenience and greater accuracy of the test itself.

A buffered diluent described by Glenny, Pope and Waddington[1] might make such a procedure feasible. They found that most diphtheria toxins diluted in the buffer maintained their toxicity for 6 weeks at room temperature and for 6 months or more at 0° C. We began a series of experiments to check their results, using a toxin which was being employed routinely in Schick test outfits.

The toxin was prepared from veal infusion made sugar free with *B. coli,* containing 2% Difco Proteose Peptone, 0.5% sodium chloride and having a pH of 7.4 after autoclaving 30 minutes at 120° C. The "Park-Williams No. 8" strain was used and the toxin harvested after incubation at 35° C. for 5 days. The toxin was 6 years old at the time of the experiments and had an M.L.D. of 0.015 cc. Two M.L.D. plus 20% excess was accurately measured into capillary tubes and the contents diluted in 10 cc. vials of isotonic salt solution containing 0.4% phenol or in 10 cc. vials of the buffered diluent (pH 8.1) prepared according to the Glenny, Pope and Waddington directions.

The diluent is a 1.5% aqueous solution of a mixture of 57 gm. crystal borax, 84 gm. boric acid, 99 gm. sodium chloride. This solution is stable on autoclaving and has a final pH from 8.0 to 8.4.

Our toxin diluted with this buffer solution showed little loss of toxicity when left at 37.5° C. for 24 hours. This test is said to indicate that the toxin would maintain its toxicity for 6 weeks at room temperature. An attempt was made to approximate the conditions which Schick toxin undergoes in actual use. Vials of toxin diluted (a) with isotonic salt solution, or (b) with the buffer solution were sent to 2 district health officers to be carried with them on their usual rounds for 2 months. A third set was placed in a car which was driven every day and another was left at room temperature in the laboratory. Some sets were placed at 7° C. and tested at monthly intervals while others were placed at 33° C. The vials after being returned from the district health officers remained at laboratory temperature. Toxicity was determined by injecting 4 cc. and 5 cc. from each vial subcutaneously into 250 gm. guinea pigs. These injections represented approximately 1.0 and 1.25 M.L.D. respectively. The results are given in the table.

---

[1] Glenny, A. T., Pope, C. G., and Waddington, Hilda, *J. Path. and Bact.*, 1928, xxxi, 133.

TABLE I.

Conditions to which diluted toxin was submitted	Diluted in Buffered Diluent			Diluted in Salt Solution		
	5 cc. pigs	4 cc. pigs	Remarks	5 cc. pigs	4 cc. pigs	Remarks
At time of dilution	3, 3, 3	4, 4, 4				See test at 7° C. 2-6 months below
37.5° for 24 hr.	6, 5, 3	4, 7, 4		S, S, S	S, S, S	No local reaction
In car and office during Nov., Dec. and Jan. (10 wks.)	3, 5, 6, 4	4, 4, 3, 4		S, S, S, S	S, S, S, S	”
Same as above plus 4 months at room temperature	4, 9	S, S,	Maximum induration	S, S	S, S	”
In car for 10 weeks, driven about 1000 miles	3	4		S	S	”
Same as above plus 4 months at room temperature	5	8		S	8	Not toxic death
Room temperature for 10 wks.	3	3		S	S	No local reaction
33° C. 10 wks.	S	S	Maximum induration	S	S	”
7° C. 2 months	2	3		2	4	
7° C. 6 months	2	3		3	4	

Integers represent days survival of pigs after injection.
S = survival.

The results agree closely with those of Glenny, Pope and Waddington even though the time at room temperature was extended a month (they, however, report one toxin which retained full strength at room temperature for 14 weeks). In the present experiments there was no apparent deterioration of the diphtheria toxin diluted with unbuffered saline in 6 months at 7° C., although Glenny *et al* state that reduction to half strength occurs in 2 months at 0° C. When kept at 37.5° C. for 24 hours the toxin diluted with saline lost practically all of its toxicity while that diluted with the buffered solution was only slightly affected.

The tests on the companion sets exposed to the conditions which the average Schick outfit would have to meet showed that for a period of 10 weeks the buffered diluted toxin maintained its toxicity while the saline diluted toxin was destroyed.

The belief that toxins which retain their toxicity for 24 hours at 37.5° C. will retain it for 6 weeks at room temperature has been confirmed. It seems certain that those buffered toxin dilutions which stand up under this 24 hour test at 37.5° C. would be entirely satisfactory for distribution in Schick outfits.

Similar experiments have been undertaken with several of our toxins and toxins from 7 other laboratories in order to ascertain if the diluent is adapted to a sufficiently large number of toxins to warrant adopting the routine dispensing of toxin ready-diluted for Schick tests. The results of these experiments will be published in full later.

4526

Concentration of Amino-Acids, Urea, and Total Non-Protein Nitrogen in Foetal and Maternal Blood of Rabbit During Pregnancy.

FRANKLIN F. SNYDER* AND F. MEREDITH HOSKINS.

From the Department of Anatomy, University of Rochester Medical School.

It has long been recognized that there are histological changes in the placenta at various stages of pregnancy. There has been little investigation, however, of the possibility that these morphological alterations are linked with coincident changes in the concentration of various metabolites, such as glucose, urea and amino-acids in the blood which bathes the foetal and maternal surfaces of the placental boundary. In recent observations, however, it has been found that the concentration of blood sugar[1] and hemoglobin[2] is strikingly different on the foetal and maternal sides of the placental barrier in the rabbit at the beginning of the last third of the gestation period; on the other hand, at term, the blood sugar and hemoglobin are found to approach much more nearly an equal concentration in the foetal and maternal blood. It is obvious that data derived from observations at term may fail to give a complete understanding of the regulatory activity of the placenta. If the previous observations in the human are viewed in this light, as for instance, the careful description by Plass[3] of the concentration of urea, amino-acids, and total non-protein nitrogen in foetal and maternal blood at parturition, and the studies of Slemons,[4] it is evident that the data are limited to observations at a single stage of pregnancy.

---

* Department of Obstetrics, The Johns Hopkins University.

[1] Snyder, F. F., and Hoskins, F. M., *Anat. Rec.*, 1928, xxxviii, 28.

[2] Zeidberg, L. D., unpublished observations from this laboratory.

[3] Plass, E. D., *Johns Hopkins Hosp. Bull.*, 1925, xxxvi, 393.

[4] Slemons, J. M., 1919, "The Nutrition of the Foetus."

In the present experiments, a series of 24 rabbits has been observed at various stages of pregnancy from the 26th day until near term, *i. e.,* the 32nd day. As summarized in the table, it has been found that the urea nitrogen is present in about equal concentration in the foetal and maternal blood; on the contrary, the amino-acid nitrogen and the total non-protein nitrogen are found consistently in greater concentration in foetal than in maternal blood.

TABLE I.

The Concentration of Amino-acid Nitrogen, Urea Nitrogen, and Total Non-protein Nitrogen of the Foetal and Maternal Blood at Various Stages of Pregnancy.

Day of Pregnancy	Diet	Amino-acid N (mgm. %)		Urea N (mgm. %)		Total NPN (mgm. %)	
		Maternal	Foetal	Maternal	Foetal	Maternal	Foetal
26	Well fed	8.6	9.7	12.4	11.6	26.7	42.2
26	” ”	8.6	13.7	17.9	18.5	34.3	49.8
26	Fasted 25 hrs.	—	—	19.7	19.2	41.2	43.3
27	Well fed	7.6	12.1	23.5	24.0	35.4	51.0
27	” ”	8.7	11.4	13.5	13.5	33.3	44.5
27	” ”	—	—	18.3	19.8	38.5	45.0
27	Fasted 24 hrs.	7.5	10.6	—	—	—	—
27	” 26 ”	—	—	23.4	20.8	43.5	—
27	” 30 ”	—	—	17.4	14.0	—	—
27½	Well fed	—	—	11.5	12.9	33.9	47.1
28	” ”	9.1	10.5	—	—	—	—
29	Fasted 140 hrs.	8.5	11.3	24.3	26.2	44.7	56.1
30	” 72 ”	7.8	11.5	34.4	26.3	60.0	76.0
30	” 72 ”	7.1	10.8	44.0	45.9	97.5	108.5
30	” 23 ”	7.5	11.0	—	—	—	—
31	Well fed	8.1	11.8	12.2	10.0	32.4	43.0
31	” ”	8.0	13.7	26.0	25.7	52.4	62.1
31	” ”	7.9	12.6	32.7	32.9	45.4	62.6
31	” ”	7.8	9.6	26.3	29.9	51.4	55.8
31	Fasted 16 hrs.	—	—	15.6	15.8	—	—
31	” 36 ”	—	—	20.7	18.8	40.5	50.4
31	” 46 ”	8.8	12.2	50.7	49.4	70.9	82.8
31	” 48 ”	—	—	20.9	16.5	—	—
31	” 70 ”	10.0	11.6	66.1	60.2	97.4	127.4

The determinations were made on whole blood in duplicate samples, according to the methods given by Folin.[5] The animals were sacrificed by a blow on the head, and the foetuses were rapidly bled from the jugulars. Filtrates obtained from the blood of all the foetuses of a litter were usually combined.

---

[5] Folin, O., *Lab. Man. Biol. Chem.,* 1923, 235, 239, 263.

4527

The Salivary Gland Poison of the Aëdes Aegypti.

EARL B. MCKINLEY.

From the School of Tropical Medicine of the University of Porto Rico under the auspices of Columbia University, New York City.

The nature of the poison contained in the salivary glands of mosquitoes is little understood. That this substance is very irritating to the human skin (and to that of other animals) has long been an established fact. Those who dwell in the tropical belt are well aware of this though the nuisance of mosquitoes is by no means limited to warm climates. Not all species of mosquitoes bite man, indeed some seem to prefer animal blood to that of man, but a human blood meal is usually acceptable if no other is available. On the other hand, there are many different kinds of mosquitoes which prefer the blood of man and in some instances refuse to partake of the blood of certain animals. When the mosquito bites, some of the salivary secretion is injected into the wound and this, in most instances, is followed by the appearance of a wheal, surrounded by an area of erythema and accompanied by intense itching. This is the characteristic reaction following the bite of the *Aëdes aegypti* and we have been interested in the substance contained in the salivary glands of this particular insect which causes this reaction.

People vary in susceptibility to this poison. Some state that mosquitoes never bother them. Others develop the most intense reactions following these bites. Still others state that they believe that they have become immune to certain mosquitoes. Several individuals have reported to us that 2 or 3 years ago, when they first came to Porto Rico, they were bothered by mosquito bites and developed intense lesions following them and that after 2 or 3 years in residence here they have gradually become "immune" to mosquitoes and seriously doubt if they are ever now bitten. The evidence seems to justify the opinion that prolonged residence in the presence of mosquitoes and exposure to their bites produces a form of immunity against the specific poison elaborated by these insects. However, we have found that this is not always true. One of our staff who is accustomed to breed the *Aëdes aegypti* in his laboratory and allow these insects to feed frequently from his arm, developed an intense reaction with the poison extracted from the salivary glands of this insect. This test, however, was made after his absence from Porto Rico for 4 months and there is the possibility that an acquired immunity to this poison does not persist for any length of time. The fact remains that certain individuals become immune to the mosquito salivary gland poison after prolonged contact with the insect and it is upon this observation that this study has been based.

In 1888 Macloskie[1] demonstrated that the mosquito has 2 salivary glands and that each of these glands possesses 3 lobes. He found that the middle lobe of each gland differs from the others in having evenly granulated contents and staining more deeply. He regarded the middle lobe as the source of the poison. Bruck[2] in 1911, extracted a poison from the bodies of mosquitoes with water, glycerine and chloroform, which he called *culicin*. He assumed that this poison came from the salivary gland and found that it produced lesions in his own skin similar to the mosquito bite itself. Furthermore he demonstrated that the poison possessed hemolytic powers. Nuttall and Shipley,[3] working with *Culex pipiens,* extracted the salivary glands from 6 sets (36 acini) with salt solution and found that their extract neither prevented coagulation nor caused hemolysis of human blood. Schaudinn[4] observed certain yeast cells in the oesophageal diverticula (sucking stomach) of the mosquito which he believed were normal and constant commensals of the insect. He further noted that the sucking stomach emptied itself from time to time due to certain convulsive contractions of the abdomen during feeding. Schaudinn was unable to produce any irritating effect by injecting extracts of the salivary glands themselves "under the skin" though he produced noticeable and characteristic itching irritation by introducing the oesophageal diverticula with their content of yeast into an opening in the skin. From these experiments Schaudinn believed that the irritating action of the mosquito is caused by an enzyme from a commensal fungus. Yorke and Macfie[5] have shown that an emulsion of the salivary gland of *A. muclipennis* agglutinates the erythrocytes of human blood but that emulsion of the stomach and ventral oesophageal diverticulum has no such action. These authors found that the salivary gland emulsion of *Culex pipiens, Theobaldia annulata, Stegomyia fasciata* and *Glossina tachninoides* do not agglutinate human red blood cells. No hemolysin was detected in any of the insects studied by them. The salivary gland emulsion from *A. maculipennis* and of *Glossina tach-*

---

[1] Macloskie, *Am. Naturalist,* 1888, xxii, 884.

[2] Bruck, *Deutsche medizin. Wochenschr,* 1911, 1787.

[3] Nuttall and Shipley, *J. Hyg.,* 1901-3, i, ii, iii.

[4] Schaudinn, *Arb. aus dem kais. Gesundheitsamte,* 1904, xx, 387.

[5] Yorke and Macfie, *Ann. Trop. Med. and Parasitol.,* 1924, xviii, 103.

ninoides possessed a definite anticoagulating substance but this was not true for *Stegomyia fasciata.* More recently Hecht[6] described some interesting experiments dealing with this subject and has collected the literature concerning it.

In our experiments we have been interested in the toxic substance contained in the salivary glands of the *Aëdes aegypti* and in the reaction this substance produces in different individuals. We have carefully dissected out over 2000 salivary glands from female *A. aegypti* bred in the laboratory. Several very potent extracts have been prepared from these glands by grinding the glands with sand in a sterile mortar and extracting with physiological salt solution. One type preparation was made with approximately 1300 glands and extracted with 15 cc. of saline. After filtering through a Berkefeld "N" filter this material was employed for "intradermal" injection in 0.1 cc. amounts. Other similar preparations were made, using various numbers of glands and smaller quantities of saline for the extraction. We have tested this material on a large number of individuals and have obtained results which fall into 3 distinct classes. (1) Those individuals who give a typical, distinct and characteristic reaction which to all appearances is the same as a bite caused by this insect; (2) Those individuals who give no reaction whatever; and (3) those individuals who give a "delayed" reaction, coming on from 8 to 18 hours following the injection. The typical positive reaction appears in from 2 to 5 minutes following the injection and consists of a wheal (¼ to ½ inch in diameter) surrounded by an erythema varying from 1½ inch to 2 inches in breadth and length. The reaction is accompanied by intense itching and persists for approximately one hour. Reactions are not obtained in susceptible individuals if the extract is injected *under* the skin but only if given intradermally. This would readily explain the negative results obtained by Schaudinn. There is a certain agreement, but by no means constant, in our results which suggests that individuals after prolonged stay in association with this mosquito, are immune to this poison. Dark skinned individuals seem to be more immune than those having fair skins. The delayed reaction is difficult to understand and we offer no explanation for this. We have been unable to immunize individuals by repeated injection of the poison; they are still susceptible to the mosquito and to the extract after 8 or 10 daily injections. Furthermore we have been unable to produce local immunity in a given skin area by repeated injections of the extract. Intravenous injection of the extract into rabbits in an attempt to produce an immune serum have failed to produce a serum which would neutralize the poison. The poison is not destroyed by freezing to —12° C. for 5 hours or by heating in a boiling water bath for 10 minutes. The extract does not produce hemolysis of human red cells or does it prevent coagulation of the blood. This is in agreement with the work reported by Yorke and Macfie on this mosquito. The extract is without effect when injected intracutaneously into guinea pigs. The nature of this toxic substance remains unknown (toxin, foreign protein, etc.) but will be the subject for future investigation.

---

[6] Hecht, *Arch. f. Schiffs u. Tropen-Hygiene*, 1928, xxxii, 561.

4528

Phloridzin Diabetes and Vital Staining.

B. M. FRIED. (Introduced by Reid Hunt.)

From the Surgical Department of the Peter Bent Brigham Hospital, Boston.

Since the introduction in experimental pathology of vital staining of animals this method has been widely employed for the study of the physiology of the macrophage (Reticulo-Endothelial) system. In a number of instances this method has been used in conjunction with bacterial infections in the experiments with the so-called blockade of the reticulo-endothelial apparatus.

The investigation to be reported concerns itself with experiments on vital staining of animals treated with phloridzin. This substance leads to an outstanding glycosuria which, according to older observers is renal in origin. Present investigations have shown that beside the kidneys chronic phloridzin intoxication also leads to marked pathologic changes in other visceral and hematopoetic organs.

From a chemical standpoint, although the intimate mechanism of the glycosuria is not clear the loss of the function of carbohydrate oxidation is considered as being almost complete; and for practical purposes the disease is regarded as being close to diabetes mellitus (Nash[1]). It is obvious that the faulty metabolism of the carbohydrates carries with it an impairment in the combustion of fats and proteins.

Chronic phloridzin intoxication is therefore from histopathologic as well as from chemical standpoints a condition in which the entire metabolic apparatus is involved.

---

[1] Nash, T. P., *Phys. Reviews*, 1927, vii, 385.

The purpose of this investigation was then to study the response of animals with this disease to vital staining.

Full grown rabbits were used in the experiments which were divided into 2 groups: 1, animals were primarily stained for a certain period of time and then were in addition "phloridzinized"; 2, animals were at first "phloridzinized" and this was later accompanied by vital staining. One per cent trypan blue diluted in physiologic sodium chloride was daily injected in the marginal ear of the rabbit's vein in the amount of 3 cc. per kilo body weight. One gm. of phloridzin Merck diluted in 1.2% of sodium carbonate or emulsified in 7 cc. of olive oil respectively was daily injected subcutaneously.

The diet of the rabbits consisted of oats and water.

Rabbits, unlike dogs, resist for a considerable length of time phloridzin intoxication: thus, some control animals received 30 gm. of the drug in a period of 30 days without any appreciable loss of weight. On the other hand, vital dye in reasonable concentrations can be administered to animals, almost indefinitely.

The present experiments have shown in the first place that when rabbits are treated simultaneously with phloridzin and vital dye they fail much more rapidly than those that receive only one substance.

Another point of interest is as follows: Older observers, and of recent investigators Fischler,[2] and Peskin[3] stated that phloridzin intoxication deprives the animal of its power to store glycogen (glycogenoprive Intoxikation). This is true when phloridzin is administered in sodium carbonate (1.2%). But when phloridzin is given in an emulsion with olive oil (1 gm. to about 7 cc. of oil) the power of the animal to store glycogen is retained, being almost equal to the normal control. But, here too, in animals sumultaneously treated with phloridzin in olive oil and vitally stained the hepatic cells show no or only slight traces of glycogen.

---

[*] I wish to express my obligations to Dr. Cyrus H. Fiske of the Department of Biologic Chemistry, Harvard Medical School, for his suggestions during the performance of this study.

[2] Fischler, F., *Beitz. z. Path. Anat. u. z. Allg. Path.*, 1927, lxxvii, 217.

[3] Peskin, A. R., *Biochem. Z.*, 1928, cii, 5.

4529

Locke's vs. Tyrode's For Perfusion of Segments of Intestine.

ROSS C. B. THORNTON AND HARRY L. LEAVITT.
(Introduced by G. E. Burget.)

From the Department of Physiology, University of Oregon Medical School.

In previous work Tyrode's solution seemed to be inferior to Locke's for the perfusion of intestinal segments. MacLeod[1] states: "Although undoubtedly superior for some perfused preparations, such as the intestine, it does not seem to be in any way superior to Locke's for the perfusion of the heart." It seemed desirable to investigate further the value of these solutions for intestinal perfusion under the ordinary laboratory procedure.

Segments of the small intestine of the rabbit and cat were used. The segments were taken well below the duodenum because the jejunum and ileum are less erratic in activity.

The solutions used were: Locke's[2] NaCl, 0.90%; KCl, 0.042%; $NaHCO_3$, 0.032%; $CaCl_2 \cdot 2H_2O$, 0.024%; dextrose, 0.1%. Tyrode's[3] NaCl, 0.80%; KCl, 0.02%; $NaHCO_3$, 0.10%; $CaCl_2 \cdot 2H_2O$, 0.02%; $MgCl_2$, 0.01%; NaH_2PO_4, 0.005%; dextrose, 0.1%. The salts were accurately weighed and the solutions freshly prepared. The same stock of C. P. chemicals was used throughout. The Magnus type of apparatus was used, the volume of which was 200 cc.

The animal was stunned, bled from the carotid artery, and the abdomen opened at once. A segment was taken well below the duodenum and from this the required number of smaller segments were cut and dropped into their respective solutions. As short a period of time as possible was allowed to elapse from the moment the animal was stunned until the individual segment was attached to its respective piece of apparatus.

The pH of the solutions was obtained by the quinhydrone electrode. When a change of pH was desired a bath of warmed fresh solution was substituted for the bath already in use. When adrenalin was used, 1 or 2 drops of 1 to 10,000 solution prepared from Adrenalin, 1 to 1,000, Parke Davis, was added to the bath at a constant point. Aeration was accomplished by forcing air through a small size glass tube at the rate of about 200 bubbles per minute.

---

[1] MacLeod, J. J. R., "Physiology and Biochemistry in Modern Medicine," 5th edition, C. V. Mosby Co., 383.

[2] Locke, F. S., and Rosenheim, O., *J. Physiol.*, 1907, xxxvi, 205.

[3] Tyrode, M. V., *Arch. Intern. de Pharm. et de Therap.*, 1910, xx, 205.

The temperature varied between 36° C. and 38° C. This change of temperature does not appreciably alter the activity of the segment.

After the segments are placed in their respective baths there is a period of inactivity, the duration of which depends upon the kind of perfusion bath and the amount of chilling to which the segment has been subjected. A segment perfused in Tyrode's or one that has cooled much below body temperature has a longer period of inactivity than a segment bathed in Locke's or a segment whose temperature has not been allowed to fall much below body temperature.

The recorded amplitude of the movements of the segments varies, of course, with length of lever arm, length of segment, etc., but these factors were controlled. The length of time a segment has been in action also affects the amplitude; early it is small and irregular, later it increases and becomes regular and after 3 or 4 hours it slowly decreases. For a given segment the amplitude is greater when perfused in Locke's than when in Tyrode's.

Shortly after the beginning of rhythmical movements the segments bathed in Tyrode's solution rapidly lose the greater part of their tonus while the loss of tonus of segments in Locke's is more gradual and less complete. If one substitutes Locke's for Tyrode's after the segment in Tyrode's has lost its tonus a portion of the lost tonus is regained and if it is now replaced into Tyrode's it will again rapidly lose the regained tonus.

If adrenalin is added when the segment is bathed in Locke's the loss of tonus is greater than when in Tyrode's, indicating that in the former the segment has greater residual tonus. The interval of depression is shorter in Locke's than in Tyrode's. If a segment in Tyrode's has been long inactive from the action of adrenalin it may be activated by substituting Locke's for Tyrode's; this does not happen if fresh Tyrode's is substituted.

A fairly constant pH was obtained, Locke's, pH 7.6, Tyrode's, pH 7.78. During the experiment due to the active aeration the pH increases to 8.05± for Locke's and 8.2± for Tyrode's. The pH was not purposely altered during the experiment by the use of chemicals but when a lower pH was desired a fresh bath was substituted for the used bath. This procedure shows that a change of pH within the limits given does not materially alter the activity of the segment.

Although there are individual differences in the segments it has been observed that as a rule those segments immersed in Locke's maintain their activity at a higher level and for a longer period of time than do those in Tyrode's solution.

The difference in effectiveness of these 2 baths was not, in our experiments, due to dextrose or concentration of magnesium chloride. Sollman[4] has recently suggested that there exists an optimum concentration of buffer substances in perfusion baths and that this concentration varies with the size of bath and its effective aeration.

Under the conditions described a segment of rabbit's or cat's small intestine perfused in Locke's solution has a shorter period of inactivity, less early irregular activity, more gradual and less complete loss of tonus, a greater amplitude of contraction, the ability for more rapid recovery from adrenalin and greater duration of activity than a segment perfused in Tyrode's solution.

4530

Reciprocal Activity of the Muscle Coats of Guinea Pig Intestine.

A. J. HOCKETT. (Introduced by C. H. Thienes.)

From the Department of Pharmacology, University of Oregon Medical School, Portland, Oregon.

Some years ago, Gayda[1] reported a coordinated reciprocal activity of the muscle coats of guinea pig intestine, but his work received little notice. More recently, Hanzlik and Butt[2] have shown a similar mechanism in the crop of the pigeon. Using Gayda's modification of the Trendelenberg method,[3] and a further modification to prevent pressure changes in the lumen of the gut, I have been able to confirm the above workers, in that 19 of 26 segments exhibited the reciprocal activity of the 2 main muscle coats; *i. e.,* contraction of the longitudinal muscle was accompanied by relaxation of the circular muscle, and vice versa. Four of the remaining 7 segments developed reciprocal activity after treatment with atropine and nicotine. The effects were the same for duodenum, jejunum, and ileum and occurred in segments from each of 7 animals. Furthermore, the reciprocal activity of the muscle coats of the small intestine was evident during the action of epinephrine, pilocarpine and barium chlo-

---

[4] Sollman, T., von Oettingen, W. F., and Ishikawa, Y., *Am. J. Physiol.,* 1928, lxxxv, 118.

[1] Gayda, T., *Arch. f. d. ges. Physiol.,* 1913, cli, 407.

[2] Hanzlik, P. J., and Butt, E. M., *Am. J. Physiol.,* 1928, lxxxv, 271.

[3] Trendelenberg, P., *Arch. exp. Path. Pharm.,* 1917, lxxxi, 55.

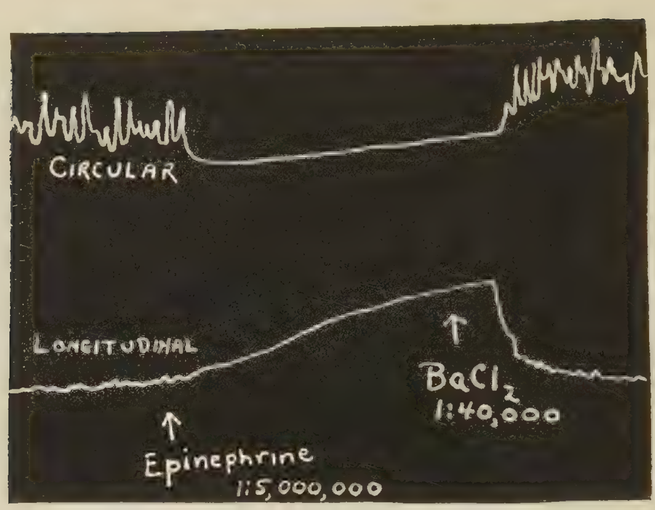


FIG. 1.

Reciprocal response of the muscle coats of the intestine to epinephrine and barium.

ride. For example, epinephrine relaxed the circular muscle and increased the tone of the longitudinal muscle; barium chloride and pilocarpine produced relaxation of the longitudinal muscle and contraction of the circular. The action of epinephrine and of barium chloride is shown in Fig. 1.

4531

A Study of the Blood of the Mother and New Born.

H. RAY ALLUMBAUGH. (Introduced by Dr. G. E. Burget.)

From the Department of Physiology, University of Oregon Medical School, Portland, Oregon.

Reports on the red cell count and hemoglobin content of the blood of the new born are at considerable variance. Williamson[1] gives the hemoglobin as 23.25 gm. per hundred cc., while others have placed the figure as low as 15.6 gm.[2] Red cell counts vary almost as widely with different investigators. This may be explained by the fact that many different methods of hemoglobin estimations have been used, and, further, to the fact that the blood of the new born changes rapidly. The steady fall in both red cells and hemoglobin in the first 2 weeks is well known. Lippman[3] pointed out a rapid rise in the blood count in the first 6 hours of life. Consequently, we considered that blood taken from the umbilical cord would give more accurate information than the common method of drawing blood at any time during the first 24 hours, especially since we desired to compare the blood of the baby with that of the mother.

---

[1] Williamson, *Arch. Int. Med.*, 1916, xviii, 505.

[2] Haden and Neff, *Am. J. Dis. Child.*, 1924, xxviii, 458.

Red cell count and hemoglobin estimations were made on 50 pregnant women at term, using oxalated venous blood obtained from a few minutes to 5 days before delivery. None of these women presented any recognized pathological condition. The baby's blood was collected in sterile oxalated tubes (dry) at the time the cord was cut. Check estimations were done and the average is the figure used. The hemoglobin was estimated by the Sahli method, using Haskin's permanent inorganic standard.

Of the 50 babies, 27 were males and 23 were females. The results were essentially the same for both sexes, so we do not report them separately.

The average red cell count of the new born was 5.52 million per cmm., with minimum count of 4.45 million and a maximum of 6.52 million. Lucas[4] reports an average of 5.51 million for 36 cases and Sanford[5] found an average of 5.8 million in 200 cases counted during the first 24 hours. The hemoglobin average of the babies was 16.85 gm. (122% Sahli). The highest in the series was 19.87 gm., the lowest 14.08 gm. A white cell count was made on 37 of the cord blood specimens. Our average count was 17,110, which does not vary widely from counts previously reported.

The red cell count on the 50 mothers gave an average of 4.21 million. The women with the lowest count had 3.29 million, and the highest had 5.23 million. The average mother's hemoglobin was 10.9 gm. (79%). The maximum was 14.9 gm. and the minimum was 7.04 gm. In a recent report, Lyon[6] found an average hemoglobin of 10.4 gm. (75.3% Sahli) on 177 women at or near term. He does not report a simultaneous check on the child's blood.

Osgood's[7] average for normal non-pregnant women was 4.8 million reds and 13.7 grams of hemoglobin. By comparison we find the mothers are deficient 600,000 red cells, or, 13% below normal.

---

[3] Lippman, *Ibid.*, 1924, xxvii, 471.

[4] Lucas, *et al.*, *Ibid.*, 1921, xxii, 525.

[5] Sanford, *Ibid.*, 1928, xxxv, 9.

[6] Lyon, *J. Am. Med. Assn.*, 1929, xcii, 11.

[7] Osgood and Haskins, *Arch. Int. Med.*, 1927, xxxix, 643.

The hemoglobin is lowered 2.8 gm. or 20%. This is the secondary anemia type.

The blood of the average baby contained 1.31 million red cells and 5.95 gm. of hemoglobin more than the blood of the average mother. Comparison of individual cases seems to present 2 types. In the case of the new born with 14.08 gm. of hemoglobin, the lowest of the babies, the mother had 9.11 gm. That is, the child is 17% below the babies' average and the mother is 14% below the average for the mothers. The child with the highest hemoglobin (19.87 gm.) is 12% above the average, and the mother of the child is 11% (12.56 gm.) above the average of the pregnant women. This suggests that the blood quality of the child may be expected to be in rather direct proportion to that of the mother. We find a similar condition in 29 (58%) of the cases. On the other hand, the mother with the highest hemoglobin (14.9 gm.) is 14% above average, whereas the child is 11% below average with 15.04 gm. In 24% of the cases there was such a change in the balance in favor of the mother. The woman with the lowest hemoglobin (7.04 gm.) is 30% below average for the mothers and almost 50% below normal. Her child, however, has a normal hemoglobin of 16.7 gm. Cases in which the balance is in favor of the child make up 18% of the series. These comparisons suggest that the "placental barrier" may function in one instance to conserve blood building elements for the mother at the expense of the child, and in another the child may be favored with material loss to the mother. This is not established by this experiment, but we hope to report further studies to clarify this point. Particular attention will be paid to cases which vary widely from the average or normal here established.

4532

Effect of Lithium, Ammonium, Strontium and Barium Ions Upon the Heart Rhythm of the Crayfish.

V. F. LINDEMAN. (Introduced by O. M. Helff.)

From the Zoological Laboratory, State University of Iowa.

The experiments on which the present observations are based are a continuation of a previous investigation (Lindeman[1]) on the effect of various ions upon the heart rhythm of the crayfish *Cambarus*

---

[1] Lindeman, V. F., *Physiol. Zool.*, 1928, i, 576.

clarkii. However, instead of employing the normal blood ions, a group of closely allied ions, found in the same periodic classification of metals were employed, namely, Li, NH_4 Sr and Ba.

The species of crayfish used was that of *Cambarus clarkii*. The same general technique was employed for obtaining graphic records as described in the above investigation. A normal perfusing solution containing 1% NaCl + 0.028% KCl + 0.024% $CaCl_2$ was employed as a control bath and as a basis for observing the effect of the solutions containing different ionic concentrations. The author has demonstrated in a previous paper that a 1% NaCl solution produces for a time a rapid decrease in tone and an increase in rate and amplitude of the beats. With continuous application, arrest is produced in about 30 minutes.

1% LiCl causes an increase in tone, followed shortly by arrest. However, upon the application of a 0.5% LiCl solution to the heart of a crayfish which has been beating normally with a normal perfusing solution, a decrease in tone immediately follows, coupled with an increase in rate and amplitude. With continued perfusing the rate and amplitude slowly decrease, with ultimate arrest in about 25 minutes. Because of the apparent similarity of 0.5% LiCl and the 1% NaCl in their action upon the heart rhythm of the crayfish, an attempt was made to replace part of the NaCl in the normal perfusing solution with LiCl. If the intact heart is perfused with a solution containing 0.3% LiCl + 0.7% NaCl + 0.028 KCl + 0.024% $CaCl_2$, it will continue to beat normally for several hours. An attempt to completely replace the NaCl by LiCl proved less successful. Upon substituting a 0.5% LiCl solution for the 1% NaCl in the normal perfusing solution, a slight increase in tone occurs, coupled with a slight increase in rate and amplitude of the beats. However, with continued perfusing the amplitude slowly decreases resulting ultimately in stoppage after a period of 45 minutes. The results obtained by the writer for crayfish compare favorably with those obtained by Hogben[2] for Homarus, demonstrating that Li is unable to completely replace Na in the perfusion media. Within certain limits, however, Li and Na are quite analogous in their effects upon the heart rhythm of the crayfish.

It is generally recognized that Sr resembles Ca in its physiological action upon tissues and is capable within certain limits of replacing Ca in some physiological processes. Upon the application of a 0.024% $SrCl_2$ solution to the intact heart of the crayfish, a sharp increase in tone immediately follows, coupled with a slight irregularity in the rhythm and ultimately arrest in about 5 minutes. Subsequent application of normal perfusing solution results, in time, in complete recovery of the normal rhythm. In order to test the further relationship of Ca and Sr in their effect upon the heart rhythm of the crayfish, a 0.020% solution of $SrCl_2$ was substituted for the 0.024% $CaCl_2$ in the normal perfusing solution. Upon the application of such a solution to the heart a slight increase in tone generally occurs, coupled with a slight decrease in rate. With continuous perfusing this rhythm will continue in some cases for as long as 5 hours. It is therefore concluded that Ca and Sr have somewhat analogous effects upon the heart rhythm of the crayfish.

---

[2] Hogben, L. T., *Quar. J. Exp. Physiol.*, 1925, xv, 263.

The power of Ba to replace Ca in this respect is undoubtedly very much more limited, although it is probably not entirely lacking. $BaCl_2$ always produces an increased tone of the heart which leads ultimately to systolic standstill.

Upon the application of a 0.024% $BaCl_2$ solution an increase in tone occurs, followed by an irregularity in the pulsations. Systolic arrest is generally obtained in less than 2 minutes. When 0.020% $BaCl_2$ is substituted for the 0.024% $CaCl_2$ in the normal perfusing solution and perfused through the heart a slight increase in tone occurs. With continued perfusion there is a slow decrease in the amplitude and force of the beats resulting ultimately in arrest in a few minutes.

NH_4Cl likewise has a depressor effect upon the heart rhythm of the crayfish. Upon the application of a perfusing solution containing 1% $NaCl + 0.040\%\ NH_4Cl + 0.028\%\ KCl + 0.024\ CaCl_2$, there occurs an increase in tone, a decrease in rate and amplitude, with systolic arrest within a few minutes. No recovery can be obtained.

4533

Inhibition of Oestrus in the Rat by Extracts of Corpus Luteum.*

H. O. HATERIUS AND J. J. PFIFFNER. (Introduced by W. W. Swingle.)

From the Zoological Laboratory, State University of Iowa.

Although no data are recorded, Pereyra[1] (on the guinea pig) and Hisaw[2] (on the rat) pointed out the fact that inhibition of oestrus was obtained by acid alcoholic extracts of *corpus luteum*, but neither

---

* The expenses of this investigation were defrayed by a grant from the National Research Council, Committee on Research in Problems of Sex.

gave details of procedure. Parkes and Bellerby,[3] working on mice, obtained an active preparation by extracting freshly dissected luteal tissue with ether. Macht, *et al.*,[4] obtained active preparations from de-fatted desiccated luteal tissue both by dilute ethyl alcoholic and by ethyl acetate extraction.

Extraction of the oestrus-inhibiting principle from *corpora lutea* by means of organic solvents has met with varying degrees of success in this laboratory. Press juice gave positive results but there was a severe local reaction and the activity rapidly disappeared. Consistent positive results were obtained with both methyl alcoholic extraction and acidified methyl alcoholic extraction of fresh luteal tissue obtained from sow ovaries. The luteal tissue was hand dissected within 8 hours after removal of the ovaries. A typical experiment is included to show the general method of extraction which has given the most striking results: 250 gm. freshly shelled *corpora lutea* are thoroughly hashed and treated gradually with 625 cc. methyl alcohol. After standing from 12 to 15 hours at room temperature with frequent agitation the mixture is strained through muslin and the glandular residue thoroughly expressed. The strained fluid is filtered and the filtrate evaporated before a fan. The residue is suspended in normal saline solution so that 1 cc. represents 10 gm. of fresh *corpora lutea*. An active extract was also obtained using methyl alcohol containing 1% acetic acid. Control extracts were prepared in the same manner from fresh muscle tissue.

Each test animal received the equivalent of 5 to 10 gm. of tissue per day administered subcutaneously. Only animals displaying normal cycles were used. Cycles were followed by means of the vaginal smear method.

Experimental dioestrous intervals ranging from 8 to 22 days were observed in 4 of the 8 test animals receiving extract prepared as described. The remaining 4 animals responded with dioestrous intervals of 6 to 13 days. Of the 8 animals on extract prepared with acidified methyl alcohol all but one responded, none of them coming into oestrus during the period of injection; control extracts did not disturb the oestrus cycle.

Further work on the purification of these extracts is in progress.

---

[1] Pereyra, A., *Anat. Rec.*, 1928, xxxviii, 57.

[2] Hisaw, F. L., *Physiol. Zool.*, ii, 59.

[3] Parkes, A. S., and Bellerby, C. W., *J. Physiol.*, lxiv, 233.

[4] Macht, D. I., Stickels, A. E., and Seckinger, D. L., *Am. J. Physiol.*, lxxxviii, 65.

4534

The Possible Oestrus-Inducing Effect of Commercial Taurocholate.*

H. O. HATERIUS, J. J. PFIFFNER AND W. O. NELSON.
(Introduced by W. W. Swingle.)

From the Zoological Laboratory, State University of Iowa.

Gsell-Busse[1] has recently reported the production of oestrus in the rat and mouse through the administration of commercial preparations of sodium taurocholate—a subcutaneous dosage of 30 mg. bringing about oestrus in about 60 cases, tested according to the method of Allen and Doisy.[2]

Striking results were also obtained in the promotion of uterine hypertrophy, both in immature and in spayed adult rats. The uteri in sexually immature rats increased from 2½ to 5½ times the size of controls, following 14 days' subcutaneous injection of 25 mg. commercial taurocholate daily. Similar results were obtained in young rabbits and guinea pigs. Precocious sexual maturity was induced in immature rats by administration of 20 mg. of taurocholate in two doses, oestrus appearing at the end of two days.

The same author also reported the prolongation of the oestrous phase in rats by repeated daily injections of 25 gm. of taurocholate. Oestrus appeared in two days and persisted throughout the period of injection—from three to six weeks in some instances.

In attempting to repeat this work the authors have been unable to confirm Gsell-Busse. Following is a brief summary of results obtained.

Production of oestrus: Five spayed adult female rats received subcutaneously 30 mg. of commercial sodium taurocholate (Merck) daily. No animal came into oestrus (tested by the vaginal smear technique)even though the injections were continued for one week. On the contrary, severe diarrhea set in on the third day and lasted throughout the course of injection. The animals developed severe local reactions at the site of injection, became "droopy", and were found upon autopsy at the end of seven days' injection to have the typical genital atrophy of castration. Another commercial preparation of sodium taurocholate (Eastman) was tried on five experimental animals, with the same results. A similar series treated with sodium glycocholate (Merck) also failed to respond.

---

* The expenses for this investigation were defrayed by a grant from the National Research Council, Committee on Research in Problems of Sex.

[1] Gsell-Busse, A. A., *Arch. f. d. ges. Physiol.*, 1928, ccxix, 626.

[2] Allen, E., and Doisy, E. A., *Am. J. Anat.*, 1922, xxx, 297.

Induction of precocious sexual maturity: A litter of eight immature (30 day) female rats was utilized; two being given sodium taurocholate, two sodium glycocholate, and four kept as controls. Subcutaneous injection twice daily of 15 mg. each failed to produce precocious sexual development, although injections were continued for eight days. Severe diarrhea set in on the third or fourth day and the rats suffered some degree of general emaciation. At the end of eight days they were appreciably smaller than the controls. Injections produced severe local reactions.

Gsell-Busse also reported an "antimasculine" effect of sodium taurocholate. He found that injections of 20 mg. daily for 19 days resulted in a marked retardation of development of the testes in young experimental animals. In attempting to repeat this experiment on rats the authors obtained essentially the same results, but the disparity in weight between experimental animals and litter controls was found not to be confined to the genitalia but was true for the animal as a whole. Typical data are tabulated below.

Four litter mate males (35 days old); two on taurocholate (Merck), two as controls. At the end of 14 days' injection of 20 mg. per day the following weights were recorded at autopsy:

Injected	body weight	left testicle	right testicle	kidney
Rat No. I	130 gm.	0.382 gm.	0.830 gm.	1.222
Rat No. II	127 gm.	0.615 gm.	0.430 gm.	
Controls				
Rat No. I	205 gm.	1.248 gm.	1.683 gm.	1.857
Rat No. II	203.5 gm.	1.133 gm.	1.135 gm.	

Essentially the same results were obtained with commercial sodium glycocholate.

It appeared obvious from the foregoing results, and also from the work of Horrall and Carlson[3] that cholate salts, instead of promoting oestrus, were actually inimical in their effect upon the animal. This suggested the following experiment.

Antagonistic effect of taurocholate to Estrogen. (Estrogen supplied by Parke, Davis & Company.) Three spayed females were given three subcutaneous injections six hours apart of Estrogen (a total of 2 rat units), along with three 15 mg. subcutaneous injections of taurocholate (Merck). Three controls received the same amount of Estrogen without being given taurocholate. The controls came into typical oestrus on the third day, whereas those which received both Estrogen and taurocholate failed to display any change.

---

[3] Horrall, O. H., and Carlson, A. A., *Am. J. Physiol.*, 1928, lxxxv, 591.

It is to be concluded that the commercial preparations of sodium taurocholate used in this study do not contain a principle similar in action to the ovarian hormone.

4535

Studies on Simple Goiter Produced by Cabbage and Other Vegetables.

DAVID MARINE, EMIL J. BAUMANN AND ANNA CIPRA.

From the Division of Laboratories, Montefiore Hospital, New York.

Chesney, Webster and Clawson[1, 2, 3] have shown that when fresh cabbage *(Brassica oleracea)* is fed to rabbits as the principal food, clinically detectable thyroid hyperplasia occurs in 2 or 3 months, and with continued cabbage feeding very large goiters (up to 45 gm.) have been produced. They further showed that the development of goiter was associated with a lowering of the metabolism and that the administration of small amounts of iodine readily raises the metabolic rate in goiterous rabbits and prevents thyroid hyperplasia. In these respects, as well as histologically, the thyroid hyperplasia produced by feeding cabbage is identical with that of simple or endemic goiter.

We have confirmed these findings.

Boiling or steaming cabbage for 30 minutes increases its capacity to produce thyroid hyperplasia. Steaming for 15 minutes is less effective and steaming for 60 minutes renders it neither more nor less potent than for 30 minutes. Steamed cabbage from which 60% of the weight is removed as press juice is practically as effective as whole cabbage when fed in calori-equivalent amounts. Hashed fresh cabbage has very little capacity to produce thyroid hyperplasia, while hashed steamed cabbage fully retains this quality. In our hands the press juice of 500 gm. of steamed cabbage (300 cc.) when fed daily to rabbits whether whole or concentrated *in vacuo* was only slightly effective.

Rabbits previously iodized and fed with fresh cabbage up to 75 calories per kg. per day (3 gm. of cabbage are equivalent to approx-

---

[1] Chesney, A. M., Clawson, T. A., and Webster, B., *Johns Hopkins Hosp. Bull.*, 1928, xliii, 261.

[2] Webster, B., Clawson, T. A., and Chesney, A. M., *Johns Hopkins Hosp. Bull.*, 1928, xliii, 278.

[3] Webster, B., and Chesney, A. M., *Johns Hopkins Hosp. Bull.*, 1928, xliii, 291.

imately 1 calorie) developed palpable thyroids in about 30 days, while with the same amount of steamed cabbage only 10 to 15 days were necessary. The lowering of the metabolic rate to the thyroidectomy level (from about 2.4 to 1.75 calories per kg. per hour) would also occur in about half the time required if fresh cabbage were used. Winter cabbage is much more effective than summer cabbage. Steamed winter cabbage is effective when fed in amounts as low as 25 calories per kg. per day. It may be effective below this level although this cannot be stated with certainty because the thyroid is very labile and, as is well known, hyperplasia may be produced by a great variety of food stuffs, cracker dust to mice (Hunt), fats to pigeons (McCarrison), liver to dogs, fowls, trout (Marine and Lenhart). Fresh or steamed carrots *(Daucus carota)* and lettuce *(Lactuca sativa)* when fed in amounts up to 75 calories per kg. per day have no thyroid hyperplasia producing qualities. Mangel roots *(Beta vulgaris,* var. *macrorhiza)* when fed either fresh or steamed have slight goiter producing qualities. Fresh turnip *(Brassica campestris* and *Brassica rapa)* is practically ineffective. If steamed for 30 minutes, turnip has a moderate goiter producing quality. Brussels sprouts *(Brassica oleracea,* var. *bullata gemmifera)* and cauliflower *(Brassica oleracea botrytis)* also produce thyroid hyperplasia approximately to the same degree as cabbage and their goiter producing quality is likewise greatly increased by steaming.

The isolation of a powerful reducing substance from cabbage and also from ox suprarenal cortex by Szent-Györgyi[4] which he identified as a hexuronic acid suggested the possibility that this substance might be a factor in the production of thyroid hyperplasia since such a reducing factor could be correlated with our often expressed conception that the suprarenal cortex contains a hormone capable of exerting a regulatory or inhibitory effect on many physiological activities including heat production. Sterile fractions containing this substance in amounts varying from 16 to 32 mgm. (as determined iodometrically) injected into rabbits intraperitoneally daily for 30 days did not produce thyroid hyperplasia. Indeed, the opposite effect (involution) occurred.

Although cabbage is an excellent food for rabbits it is evident that it contains a powerful goiterogenic agent which can be destroyed by enzymes existing in the plant and which can be preserved for many days when these enzymes are destroyed by heat. Like other goiterogenic agents it exhausts the thyroxine store in the thyroid and hyperplasia follows when this exhaustion reaches the critical level. The thyroid hyperplasia is therefore due immediately to a relative deficiency of iodine and an iodine store sufficient to maintain normal thyroid structure for from 3 to 6 months may be exhausted in 2 weeks by feeding steamed cabbage. The possibility that the thyroid hyperplasia due to feeding cabbage is dependent upon an absolute iodine deficiency can be dismissed.

---

[4] Szent-Györgyi, A., *Biochem. J.,* 1928, xxii, 1386.

The mode of action of this goiterogenic agent is unknown, although the data available suggest that it acts by depressing some oxidation system which the thyroid attempts to overcome.

4536

Contribution to the Problem of the Origin and Development of the Sympathetic Nervous System.

ERNEST VAN CAMPENHOUT. (Introduced by H. S. Burr.)

From the Department of Anatomy, Yale University School of Medicine.

Three theories have been proposed regarding the origin of the sympathetic elements, the neural crest, the ventral half of the spinal cord, or the mesodermal tissue. The experimental method alone is able to give a satisfactory answer to this problem.

In embryos of *Rana pipiens,* at the stage of open or closing neural folds, the author has removed the neural crest from the hindbrain to the tip of the tail; the entire spinal neural crest and the dorsal half of the spinal cord were excised by that procedure. Many operated embryos live as long as 45 days after the operation. Their size was very much reduced if compared with the control animals and they showed marked deficiency in their sensitivity. The microscopic study of these embryos shows the absence of the spinal and sympathetic ganglia throughout the entire trunk region. The motor roots are present in all segments but are lacking sheath cells. The spinal cord shows normal motor neurones; the Rohon Beard elements are absent and the central canal is more or less dilated. The chromaffin part of the adrenals is undeveloped and the digestive tube does not show any nervous elements in its walls.

The removal of the hindbrain at the same embryonic stage does not interfere with the development of a vagal ganglion. Its visceral branch is, however, absent. The visceral portion of the vagus nerve seems to be exclusively derived from the medullar neural crest, the other components originating from the placodes. In these embryos the central and peripheral parts of the sympathetic system are well developed; the adrenals show their 2 constituting anlagen and the intestinal walls contain nervous elements.

The 2 experimental series strikingly confirm each other and they show that, in the frog, the neural crest represents the exclusive source of sympathetic elements, that the visceral branch of the vagus nerve is derived from the medullar neural crest and that the nervous elements in the intestinal walls are to be related to the sympathetic system.

The study of a series of normal chick embryos, stained with ordinary techniques or impregnated by various silver methods, gives very clear pictures concerning the different points of the development of the sympathetic system. There is no evidence of a succession of 2 independent sympathetic chains. Around the sixth day of incubation, the communicating rami become shorter, thus probably corresponding to a neurobiotactic phenomenon resulting from the penetration of motor fibers into the sympathetic ganglia. The exclusive origin of the elements of the sympathetic chains seems to be the ganglionic crest and no migration was found along the ventral roots. The vagus nerves supply the primary innervation to the lungs, to the heart and to both gastric pouches. The small intestine, from the pylorus on, is innervated primarily by sympathetic branches and there is no evidence of any participation of the vagus nerves as late as the eighth day of incubation, when the intestinal walls show a perfectly developed nervous supply with myenteric and submucous plexuses.

The neural crest origin of the sympathetic elements and the sympathetic origin of the intestinal nervous structures are the main conclusions of this contribution.

4537

I. Effect of Intravenous Injections of Sodium Sulphide and Sodium Cyanide on Reflex Response of Anterior Tibialis Muscle.

HAROLD H. GAY. (Introduced by Robert Gesell.)

From the Department of Physiology, University of Michigan.

Glazer[1] has shown that intravenous injection of sodium cyanide increases the reflex response of the anterior tibialis muscle to electrical stimulation. The purpose of this group of experiments was to make a similar comparison of the effects of intravenous injections of sodium sulphide on reflex response and respiratory movements.

---

[1] Glazer, W., *Am. J. Physiol.*, 1929, lxxxviii, 562.

The anterior tibialis muscle was isolated, its insertion severed and secured by a thread to a writing lever. A large copper indifferent electrode was fastened to the shaven side of the neck, and a sharp point electrode thrust into the plantar surface of the foot. The trachea was connected to a rebreathing tank for a recording respiration.

An increase in reflex response always followed an injection if it were large enough. Forty-four injections of varying amounts were given to 17 dogs. Two injections caused an increase before hyperpnea had begun, 8 increases began with hyperpnea, and ended with it, 10 began during hyperpnea and lasted into apnea, 12 began during hyperpnea and lasted through apnea and 4 began after hyperpnea and lasted through apnea. Eighteen injections were followed by no apnea. Fatal injections always caused a pronounced increase in reflex response.

Since hyperpnea leads to increased elimination of carbon dioxide it was essential to determine whether the increased reflex response was of this origin. This was accomplished by repeating the above experiments during constant artificial ventilation after the establishment of pneumothorax. The results of 21 injections on 10 animals were comparable to those obtained in the intact animal with ventilation under normal control.

4538

II. Low Alveolar Oxygen and Response of Anterior Tibialis Muscle to Reflex and Motor Nerve Stimulation.

HAROLD H. GAY. (Introduced by Robert Gesell.)

From the Department of Physiology, University of Michigan.

Glazer and Winkler[1, 2] have shown that the administration of low percentages of oxygen in nitrogen under normal conditions of respiration caused an associated increase in reflex response and respiratory movements. The augmented reflex response might be due to the blowing off of carbon dioxide by the augmented respiration. To investigate this possibility a group of 6 experiments were done in which low percentages of oxygen (4% to 7%) in nitrogen were

---

[1] Glazer, W., *Am. J. Physiol.*, 1929, lxxxviii, 562.

[2] Winkler, A., *Am. J. Physiol.*, in press.

administered under conditions of constant ventilation as well as normal variable ventilation.

It was found that low alveolar oxygen during constant pulmonary ventilation augmented the reflex response of the anterior tibialis muscle similar to that during normally augmented ventilation.

To determine the site of action of low oxygen pressure, both anterior tibialis muscles were isolated and stimulated, one reflexly as above and the other through its severed motor nerve. In the 4 experiments that were done it was found that the response to the direct stimulation of the motor nerve was the same as the reflex response. Whether the change in reflex response is due entirely to the action of low oxygen pressure on the motor end of the reflex arc remains to be determined.

4539

III. Varying Degrees of Ventilation and Response of Anterior Tibialis Muscle to Reflex and Motor Nerve Stimulation.

HAROLD H. GAY. (Introduced by Robert Gesell.)

From the Department of Physiology, University of Michigan.

Seventeen experiments were performed in which carbon dioxide mixtures were administered by artificial ventilation. In 11 of these the carbon dioxide percentage was successively increased from 5 to 25%. In 15, ventilation with pure oxygen was varied from under ventilation to over ventilation, thus permitting large fluctuations in alveolar carbon dioxide pressure without undue lowering of alveolar oxygen pressure.

Carbon dioxide reduced the reflex response of the anterior tibialis muscle in all but one experiment. It reduced nerve-muscle response in all but 5 of the 17 experiments. In some experiments even high carbon dioxide mixtures did not depress the motor nerve response, whereas the reflex response was decreased.

Under ventilation in oxygen reduced reflexes in all but 2 experiments. It reduced nerve-muscle responses in all but 8 of the 17 experiments. Pure oxygen never failed to restore reflexes after carbon dioxide in pure oxygen had reduced them. Over ventilation always restored reflexes after they had been depressed by under ventilation in pure oxygen. Pure oxygen occasionally caused hypertonus, particularly in the muscle with the nerve intact, and on 3 occasions in the other muscle as well. Where pure oxygen was administered after room air the reflex response frequently fell off somewhat.

4540

IV. Irregular Respiration, and the Response of Tibialis Muscle to Reflex and Direct Motor Stimulation.

HAROLD H. GAY. (Introduced by Robert Gesell.)

From the Department of Physiology, University of Michigan.

In the course of the series on reflex response of the tibialis anterior muscle several animals were found in which the respiration was very irregular. There were periods of hyperpnea followed by varying periods of apnea. In 3 experiments in which periodicity occurred during the administration of room air, hyperpnea was accompanied by increased reflex response and apnea, especially when profound and long continued, by periods of reduction or total disappearance of reflex response. In one experiment periodicity occurred during both the administration of room air and of 7% oxygen in nitrogen. The effect here was the same as in the 3 experiments above. In another, irregular respiration occurred while the animal was breathing room air and while breathing pure oxygen. Again the results were the same. The response of the muscle to direct motor nerve stimulation was similar to the reflex response of the opposite muscle.

From these and the preceding experiments it seems probable that there is no single point in the system which can be said to control the response of the anterior tibialis muscle to sensory stimulation. The evidence seems, rather, to suggest that the response may be the result of a number of factors which may vary individually. On the whole, with the exception of carbon dioxide, the reflex response and the response of the tibialis muscle to direct motor nerve stimulation varied roughly with the magnitude of respiratory movements.

4541

Cerebral, Head and Body Flow of Blood During Periodic Ventilation.

PAUL O. GREELEY AND CHARLES E. GREELEY.
(Introduced by Robert Gesell.)

From the Department of Physiology, University of Michigan.

Periodic ventilation was produced in dogs under morphine-urethane anesthesia by (a) increased intracranial tension and (b) blocking of cerebral arteries. Practically complete control of head flow was obtained by ligating the vertebral arteries, and anterior spinal arteries and small arterioles of the cord, all in the region of the second cervical vertebra. All muscles at this level were severed. The vagi were left intact and one carotid was used for volume flow measurements. Blood volume flow changes were followed in the carotid and femoral arteries by the thermo-electric method (Gesell and Bronk.[1] The external jugular, vertebral, and femoral venous return was measured by the enclosed drop method; the submaxillary vein by the external drop. Blood pressure, ventilation, oxygen consumption, and time were simultaneously registered with the blood volume flow changes.

Two types of respiration were obtained. Type 1 gave typical symmetrical Traube Herring blood pressure waves whose frequency was that of the respiratory waves. Apneas were either short (2 seconds) or lacking; respiration in other characteristics was quite normal. The depth of respiration decreased as the carotid flow increased, sometimes to the extent of apnea; as the flow decreased to a minimum, respiratory depth increased to a maximum. The respiratory depth again decreased as the flow increased. Type 2 was characterized by very pronounced blood pressure changes which were not typical Traube Herring waves. Apneas occurred. An apnea of 123 seconds was obtained in one animal during periodic breathing. The respiratory groups usually begin during some phase of the decreased flow or they may begin as late as the very end of decreased flow. Increasing respiratory movements may or may not carry on into the period of increasing blood flow. These increasing respiratory movements may suddenly give way to apnea which is associated with a high flow of blood.

4542

Acid Changes in pH of Portal, Hepatic, and Arterial Blood on Administration of Sodium Cyanide.

GEORGE MC CLURE. (Introduced by Robert Gesell.)

From the Department of Physiology, University of Michigan.

In view of the fact that the liver reconverts lactic acid into glycogen (Himwich[1]), changes in acidity of blood as it passed through the liver were studied in a series of experiments undertaken on dogs. Following the intravenous injection of sodium cyanide, which leads to excessive appearance of lactic acid in the blood, samples of blood were taken from the portal vein, hepatic vein, and femoral artery. Two sets of samples were taken at 10 minute intervals to determine the normal acidities, and 2 sets of samples were taken at 10 minute intervals after each injection of sodium cyanide. The blood samples were stored on ice in special pH tubes designed by Dr. Gesell, and analyzed immediately following the experiment by the quinhydrone method.

---

[1] Gesell, R., and Bronk, D., *Am. J. Physiol.*, 1926, lxxix, 61.

[1] Himwich, H. E., *J. Biol. Chem.*, 1923, lv, 525.

Constant results on 12 curves showed that: 1. Normally portal blood was more acid than hepatic blood. 2. Normally arterial blood was more acid than hepatic blood. 3. Portal blood became markedly more acid after each injection of sodium cyanide, then slowly returned towards normal again. 4. Arterial blood became more alkaline after each injection of sodium cyanide, then slowly returned towards normal again. 5. Hepatic blood became slowly but steadily more acid through the entire experiment. The absence of a marked rise in acidity of the hepatic blood could not be explained by the increased alkalinity of the arterial blood.

This flattening of the hepatic acidity curve may, therefore, be due to the fact that a portion of the surcharge of lactic acid causing the abrupt acid rise of the portal blood is reconverted by the liver into the precursor state (glycogen), with the liberation of free base. It is, therefore, suggested that the reconversion of lactic acid into glycogen by the liver is a factor in the maintenance of the acid-base control of the body.

4543

Effect of Low Oxygen on Muscular Contraction.

R. R. GETTEL AND L. F. KNOEPP. (Introduced by Robert Gesell.)

From the Department of Physiology, University of Michigan.

This study deals with effects of lowered alveolar oxygen on muscular activity of the dog. Twin rebreathing tanks were used containing room air and low oxygen mixtures respectively. Supermaximal stimuli were applied with an automatic stimulator to the peripheral end of the sartorius muscle whose motor nerve was severed to insure direct muscular stimulation. The results of 3-5% mixtures in most animals revealed increased respiration with a corresponding increase of amplitude of muscular response, this passing into a gradual decline to nearly zero with the continuing but declining hyperpnea. The subsequent administration of room air gave a decided rise of muscular activity to a nearly normal level of contraction accompanied by the usual respiratory changes. When the condition of the animal became more critical, post administration level was gradually lowered below previous starting levels. In some animals both the muscular and respiratory responses were well augmented over long periods of administration of low oxygen mixtures. Other animals gave no initial rise in muscular activity. The muscular response gradually decreased until room air was administered though the usual hyperpnea prevailed.

It is suggested that the augmented respiratory movements of lowered alveolar oxygen in most of the observations may be partly accounted for by peripheral chemical changes occurring within the respiratory muscles themselves. In other cases the hyperpnea was maintained despite decreased muscular response to direct stimulation.

4544

Effects of Low Alveolar Oxygen and High Alveolar Carbon Dioxide on Rate of Flow of Cerebro-Spinal Fluid of the Dog.

HAYDEN NICHOLSON. (Introduced by Robert Gesell.)

From the Department of Physiology, University of Michigan.

The fluid was obtained either by placing a cannula in the *cisterna magna* or by lumbar puncture, in most cases the former method being used. The rate of flow was recorded by the drop method.

High percentages of carbon dioxide in room air (8-14%) invariably increased the rate of flow, in one case reaching 1000%. This increase appeared promptly on administration of the gas, rapidly increased to a maximum, and persisted practically undiminished up to the readministration of room air, when the flow quickly returned to the preadministrational rate.

The effects of low oxygen are somewhat more complex. Low oxygen almost invariably caused a decrease in flow, followed, after prolonged administration, by a gradual increase, approaching, but practically never reaching, the preadministrational rate. The initial decrease in flow was usually preceded by a slight and very brief increase. When room air was again administered there was usually a brief but distinct decrease in flow, followed by a marked increase above the rate during administration of low oxygen and approaching the preadministrational rate.

These results seem to be in agreement with those obtained by Eddy[1] on salivary secretion. The effects of high carbon dioxide resemble the results of Gesell[2] on the rate of flow of lymph but the resemblance does not hold for low oxygen.

4545

Effect of High Oxygen Pressures on Blood Acidity, Oxygen Consumption, Volume Flow of Blood and Respiration.

JOHN W. BEAN. (Introduced by Robert Gesell.)

From the Department of Physiology, University of Michigan.

With the purpose of throwing new light on the causes of hyperpnea as seen in animals breathing pure oxygen at high pressures, apparatus was devised whereby respiration, blood pressure, blood acidity changes, and blood volume flow could be recorded on smoked paper, with the experimental animal exposed to increased oxygen pressure.

A Hutchinson type spirometer was used; the bell counterbalanced by a solenoid around a glass tube; the writing point attached to a moveable iron core in the bore of the tube which was open to atmospheric pressure. Blood pressure was recorded in similar manner, the solenoid floating on the mercury; heart rate by an electrical contact device on the femoral artery; blood acidity changes by the continuous method (manganese dioxide electrode); volume flow of blood changes by the thermo-electrical method.

The experimental animals were dogs of from 5 to 12 kg., anesthetized with morphine sulphate and urethane. The pressures used were from 3 to slightly less than 5 atmospheres pure oxygen.

The results indicate an increase in the acidity of the arterial blood and decrease in the heart rate of the animals under high oxygen pressures. The oxygen consumption of animals under pressure showed some variation but the weight of the evidence pointed to a decrease in metabolism with a tendency to return after decompression to atmospheric pressure. In several experiments periodic respiration with an accompanying periodicity in the volume flow of blood in the common carotid artery was obtained. Hyperpnea was not invariably a response to high oxygen pressure exposure. On the contrary some individuals showed a decrease in respiratory rate to the extent of apnea.

---

[1] Eddy, N. B., *Am. J. Physiol.*, 1929, lxxxviii, 524.

[2] Gesell, R., *Am. J. Physiol.*, 1929, lxxxviii, 546.

4546

Peripheral Chemical Control of Pulmonary Ventilation.

CHARLES R. BRASSFIELD. (Introduced by Robert Gesell.)

From the Department of Physiology, University of Michigan.

To gain further evidence on the probability of peripheral chemical control of pulmonary ventilation due to pulmonary vagal stimulation, a study was made of the latent periods and types of response following injections of sodium cyanide and sodium carbonate both in the normal and vagotomized dog. Injections were made: 1. Intravenously at the level of the heart by means of a catheter inserted into the right femoral vein or right external jugular. 2. Intra-arterially in the aorta proximal to the nutrient bronchial arteries by means of a catheter inserted upward through the left femoral artery.

Dogs under morphine and urethane anesthesia were used and respiratory movements recorded with a spirometer. 514 injections varying from ¼ cc. to 5 cc. of M/100 NaCn and from 5 cc. to 20 cc. of M Na_2CO_3 were made on 21 different animals.

The latent periods of respiratory stimulation following intravenous injections in the intact animal and corresponding injections with the same animal vagotomized were for the most part the same. In all injections the latent periods were long enough to allow the injected substances to reach the respiratory center.

Intra-arterial injections just proximal to the nutrient bronchial arteries produced respiratory effects only after large amounts had been injected. The latent periods were rather long and were the same in the intact and vagotomized animal. These results are indicative of an absence of action on the pulmonary vagal endings. However, this does not preclude the findings of Heymans and Heymans[1] that the heart and aortic arch are the seat or origin of respiratory reflexes.

The responses to intravenous and nutrient bronchial arterial injections were of the same type in both the vagotomized and intact animal as those resulting from small injections into the innominate artery, thus suggesting central action as predominant in all injections.

The similarity of results obtained by administration of sodium cyanide and of sodium carbonate before and after double vagotomy together with a failure to establish a difference in latent periods before and after vagotomy suggests the absence of or a slow response to peripheral chemical stimulation of the pulmonary vagal endings.

---

[1] Heymans, J. F., and C., *Arch. Int. Phar. et Ther.*, 1927, xxxiii, 366.

4547

A Comparison of Synchronous Experimental Changes in Respiratory and Cardiac Rhythm of the Dog.

ROBERT GESELL AND JOHN NYBOER.

From the Department of Physiology, University of Michigan.

This study attempts to establish the degree of similarity of response of 2 rhythmically discharging systems to similar changes in environmental conditions with the tentative assumption that similar response is indicative of a similar fundamental mechanism operating in the pace setter of respiration and the pace setter of the heart. The subject of respiratory rhythm might thus be put on a more fundamental basis and become susceptible to a more direct analysis.

Lowered alveolar oxygen and intravenous injection of sodium cyanide and sodium sulphide augmented respiratory and cardiac rate in a comparable way. Comparable changes in rate were obtained after section of the vagus nerves and removal of the stellate ganglia.

Administration of carbon dioxide almost invariably slowed the heart. When administered in pure oxygen, though the magnitude of respiratory movements was always increased, there was either slight or no reduction in rate or slight acceleration. When carbon dioxide was administered in a gaseous mixture containing approximately 22% of oxygen respiratory rate was usually augmented. The results were similar after vagotomy and removal of the stellate ganglia.

In view of the similarity of action of lowered alveolar oxygen and of sodium cyanide and sulphide, and the common tendency towards retardation on the administration of carbon dioxide, it is tentatively assumed that there is a common mechanism of rhythmic discharge in the respiratory and cardiac rhythm centers. Granting that increased intracellular acidity is a controlling rhythm factor it is concluded that there are auxiliary factors as well. Since cyanide and sulphide may decrease the hydrogen ion concentrations of the immediate external environment and alter the state of oxidation of the neurone membrane they may so exert their effects. This agrees with suggestions offered in our electro-chemical hypothesis of respiratory control.[1]

---

[1] Gesell, R., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 263.

4548

Inorganic Elements of Spinach in Treatment of Nutritional Anemia.

HELEN S. MITCHELL AND LILA MILLER. (Introduced by H. B. Lewis.)

From the Nutrition Laboratory, Battle Creek Sanitarium, Battle Creek, Mich.

The factors necessary for the synthesis of hemoglobin in the animal body have been attracting increasing interest since the announcement by Hart and Steenbock[1] that copper seemed to play a catalytic rôle in this connection. In attempting to determine whether other inorganic factors played a similar rôle, it occurred to us to try once more the combination of minerals naturally present in a food generally conceded to be a good blood builder. A concentrated water extract of spinach was fed in quantities sufficient to supply 0.5 mg. Fe per day as supplement to the milk diet. It had previously been determined that this extract contained copper in appreciable amounts, Fe : Cu : : 37 : 1. It was to be expected that hemoglobin synthesis on spinach extract would be rapid, but to our surprise the response was almost twice as rapid and the ultimate level of hemoglobin reached and maintained was 25% higher than the response on the iron and copper combination.

This led to the question whether there might be an organic factor involved as has been so often suggested. The extract was dried and ashed and the ash fed to rats in equivalent quantities, *i. e.*, 0.5 mg. Fe per day. The ash was not completely soluble, therefore daily feedings were weighed out in the dry state and mixed with a few cc. of milk which was fed to the rats when hungry. No more milk was fed until the dishes were licked clean. In spite of this precaution to make sure that the animals received their full amount of ash, they failed to show as rapid hemoglobin synthesis as the rats on the original spinach extract. This observation made us question again the possibility of an organic factor. Previous experience[2] suggested that the insoluble nature of this ash might have some bearing on the problem. Consequently another group of animals were fed the same amount of ash which had been dissolved in a minimum of concentrated HCl which solution was then diluted to such an extent as to make it suitable for feeding. When the ash solution was used as supplement to the milk ration, a prompt and rapid response in hemoglobin was observed, equal in nearly every case both in rate and ultimate height to that obtained on the original extract.

---

[1] Hart, Steenbock, *et al.*, *J. Biol. Chem.*, 1928, lxxvii, 797.

[2] Mitchell and Vaughn, *J. Biol. Chem.*, 1927, lxxv, 123.

These results were further confirmed by feeding the extract and its ash in half the previous doses, *i. e.,* 0.25 mg. Fe per day under which circumstances differences in efficiency would be accentuated because the amount fed is well below the optimum requirement. The results, however, were entirely similar to the first series.

It is concluded that the inorganic combination naturally present in spinach is better than any combination of inorganic salts we have been able to find, *i. e.,* iron and copper, iron and copper manganese, etc. Furthermore, it would seem that the solubility of at least some of the factors present may have a real significance. A complete qualitative and further analysis of this spinach extract ash are the next steps in the investigation and will be carried out in the near future.

4549

Studies on Stimulation in Nitella.

L. R. BLINKS, E. S. HARRIS AND W. J. V. OSTERHOUT.

From the Laboratories of The Rockefeller Institute for Medical Research.

Waves of negativity occurring spontaneously in *Nitella,* sometimes in regular rhythm, have been previously described.[1] Similar negative variations, passing down the cell, may be induced in a variety of ways, by touching, bending, by application of solutions or by small direct[2] potentials.[3] They resemble the action currents of muscle and nerve in form and magnitude but last longer and are propagated more slowly.

Nitella appears to follow in general the laws of stimulation of muscle and nerve but there are some interesting exceptions. For example, it is far from obeying the all or none law completely since the P.D. (normally positive[4] when the cell is in contact with dilute solutions) may disappear partially or completely at the stimulated spot, and conduction with a decrement occurs.[5] The normal P.D. returns in from 5 to 30 seconds. The electrical (polarization) resistance falls off simultaneously with the P.D. but it takes much longer to return to normal. The period during which recovery takes place constitutes the refractory period after which the cell may again be stimulated.

---

[1] Osterhout, W. J. V., and Harris, E. S., PROC. SOC. EXP. BIOL. AND MED., 1928-29, xxvi, 124.

[2] The production of negative waves by induction shocks in *Nitella* was apparently first reported by Hörmann (Hörmann, G., Studien über die Protoplasmaströmung bei den Characeen, Jena, 1898).

[3] These are ordinarily effective at cathodal closing and anodal opening but may be induced at anodal opening by higher voltages.

[4] The protoplasm is regarded as positive when the positive current is outward, *i. e.,* when the positive current tends to flow from the inner surface through the protoplasm to the outer surface or from the outer surface through the electrometer and thence through an (imaginary) capillary passing through the protoplasm into the sap.

The stimulus is transmitted along the cell[6] at a rate of from 1 to 2 cm. per second and may pass across the "node" to a neighboring cell (or even through two aqueous contacts for a distance of half an inch or more to another cell, *e. g.,* to a cell from a different plant). The fact that it is unable as a rule to pass a spot whose positive P.D. has been (reversibly) lowered to a sufficient extent (*e. g.,* by an opposed potential or by the application of 0.05 M KCl) lends support to the idea that the wave of stimulation consists of a local current flowing outward through the protoplasm and through the cell wall to a neighboring spot which is less positive (and then back through the protoplasm and sap). Sufficient flow of current causes[7] the more positive spot to become less positive and to lose much of its electrical resistance, and a flow then starts from neighboring positive regions toward this spot and in this way the wave moves along the cell as postulated by the local action theory of nervous transmission.

Since recovery can take place while stimulation (chemical or electrical) continues it would seem that there are two opposing processes so that when the process producing a loss of P.D. (and of resistance) has reached a certain point the other automatically becomes dominant and restores the normal condition.

In many cases the negative wave has two maxima (*i. e.,* the stimulated spot becomes negative, then less negative, then more negative, after which the curve approaches zero[8]); this might be regarded as indicating that the inner layer of the protopolasm is affected before the outer. The behavior of the cell when the external solution is varied indicates that stimulation usually starts with the inner layer.[9]

---

[5] This does not necessarily mean that the protoplasm loses all its E.M.F. but only that the E.M.F. of the outer layer becomes equal to that of the inner.

[6] Waves due to cutting (death waves) travel much faster and produce irreversible changes: they differ in being able to pass a spot in contact with 0.1 M KCl or one killed by chloroform.

[7] This might be due to a structural change (production of leaks) or to an increase in conductivity (*Cf.* Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.,* 1927-28, xi, 673). Ordinarily an outward flow of positive current is more effective than an inward flow.

[8] Approaching zero merely means that the stimulated spot is recovering its normal P.D.

4550

Bioelectrical Aspects of the All or None Law.

W. J. V. OSTERHOUT AND E. S. HARRIS.

From the Laboratories of The Rockefeller Institute for Medical Research.

If the all or none law holds, a muscle fibre or nerve fibre will always give the same response when stimulated so that the total electrical effect should be directly proportional to the number of fibres which react, provided the arrangement is in series rather than in parallel. But although the fibres (in a nerve or in such a muscle as the sartorius of the frog) may have a parallel arrangement they appear to behave as if they were in series since the current of action appears to increase with the number of stimulated fibres; so also cutting a muscle entirely in two gives more current of injury than when we cut only part way across.

This is explainable if we assume (as Gasser and Erlanger[1] have done in the case of nerves) that the inactive or uncut fibres act as shunts to the E.M.F. produced by those which are active or cut. Suppose, for convenience, that a group of 5 fibres may be repre-

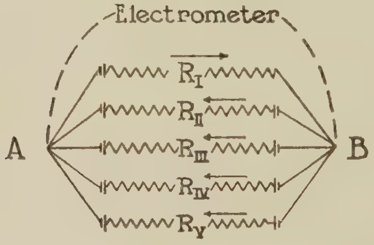


FIG. 1.

Hypothetical diagram to show electrical conditions in a group of fibres of which the uppermost (I) has been cut at the right end so that it produces the current shown by the arrows: the other fibres (II, III, IV, and V) produce no current (since the batteries in each are equal and opposite) but they act as shunts to I.

---

[9] Evidence will shortly be presented which indicates that the fibres of muscle and nerve may have a surface consisting of two layers, which is in harmony with the fact that the negative wave of nerve often shows two maxima and might afford a different interpretation from that given by Erlanger and Gasser (*Am. J. Physiol.*, 1927, lxxx, 522).

[1] Gasser, H. S., and Erlanger, J., *Am. J. Physiol.*, 1927, lxxx, 522.

sented as in the diagrams[2] in Fig. 1, where I represents a fibre cut at the right end and producing a current as shown by the arrows. Since in II, III, IV, and V the batteries are equal and opposite, these branches of the circuit contribute no current and act only as shunts (these may be called neutral and I may be called positive). We then have[3] $P.D. = E_1 R_N \div (R_N + R_P)$, where E_1 is the electromotive force of the battery in I, R_N is the resultant resistance of the neutral fibres taken together, and R_P is the resistance of the positive fibre (or the resultant resistance of all positive fibres taken together when there are more than 1). If each resistance equals R_0 we have $R_N = R_0 \div N$ and $R_P = R_0 \div P$, where N is the number of neutral fibres. We may then write[4] $P.D. = E_1 \left[\frac{R_0}{N} \div \left(\frac{R_0}{N} + \frac{R_0}{P}\right)\right] = \frac{E_1 P}{N + P}$. Putting $E_1 = 0.5$ we have $P.D. = \frac{(0.5)\ (1)}{4 + 1} = 0.1$.

When 2 fibres are cut the P.D. = 0.2; when 3 are cut the P.D. = 0.3, and so on. In other words, each fibre that is cut adds 0.1 to the P.D. as if the fibres were in series instead of in parallel.

To what extent we are justified in representing the situation in this way must be learned by experimental test. It is desirable for this purpose to know the P.D. produced by each fibre or cell as well as that of the entire group. Since this cannot readily be tested in muscle and nerve it may be worth while to record some experiments performed (incidental to other work) on the long multinucleate cells of the fresh-water plant *Nitella*. A group of these cells connected together at each end by a piece of moist cotton behaves like a group of fibres in a muscle in that the current of injury produced by cutting increases as more are cut. Here, however, we are able to measure the P.D. produced by each separate cell and to determine the effect produced by the uncut cells which act as shunts.

Experiments with Nitella.

Unless otherwise stated the technique is that described in previous papers.[5] The cells were allowed to dip into a common beaker at the right and into separate beakers at the left. In each beaker was a calomel electrode: a series of switches was so arranged that the cells could be measured singly or in any desired combination.

---

[2] In the muscle the fibres are not insulated from each other but this would not affect the result except in the case of active or cut fibres and here the effect would probably be small.

[3] It is assumed that the resistance of the measuring instrument is very high.

[4] When there are two or more positive fibres this formula holds as may be shown by setting up the usual Kirchoff equations.

[5] Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927-28, xi, 673; 1928-29, xii, 167. In the present experiments the temperature ranged from 18° to 20° C.

Since the P.D. produced by cutting changes rapidly and is highly variable it seemed better to produce a more steady and uniform P.D. by using different concentrations at the opposite ends of the cell.[6] At the start with 0.01 M KCl at both ends the P.D. (average of 17 groups of 5 cells) was practically zero. With 0.001 M KCl at one end and 0.01 M KCl at the other the P.D. was 50 millivolts (average of 17 groups of 5 cells) the end in contact with 0.001 M KCl being positive. When 1 positive cell was shunted with 4 neutral cells the P.D. was 10 millivolts.[7] In other words, the shunting effect of the 4 neutral cells (cells with the same solution at both ends) reduced the P.D. of the positive cell (which has 0.001 M KCl at one end and 0.01 M KCl at the other) from 50 to 10 millivolts, as our calculations lead us to expect.

Two cells taken together gave 49 millivolts;[8] the shunting effect of the other 3 neutral cells (III, IV, and V) reduced this to 19 millivolts. We expect 20 millivolts, so that the agreement is fairly close.

When 3 cells were positive they gave (taken together as a group of 3) 46 millivolts[9] which was reduced by the shunting effect of 2

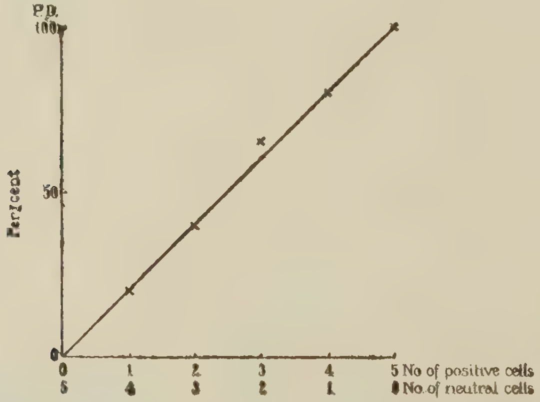


FIG. 2.

The straight line shows the calculated and the crosses the observed P.D. for each group. A group consists of 1 or more positive cells shunted by neutral cells: as the number of positive cells increases the P.D. increases as though they were in series. The ordinates represent the P.D. expressed as per cent of the P.D. observed when the shunting cells are absent.

---

[6] Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1928-29, xii, 761.

[7] Average of 17 groups of 5.

[8] Average of 17 groups of 2.

[9] Average of 17 groups of 3.

neutral cells to 30 millivolts; in this case the expectation[10] is 28 millivolts.

When 4 cells were made positive they gave 48 millivolts;[11] on shunting with a fifth cell this was reduced to 38 which agrees with the prediction.[12]

The results are shown graphically in Fig. 2. Measurements were also made by shunting cells in other groupings. Here also the results were in accordance with prediction as is shown in Fig. 3.

As an analogy to action currents we may consider the effect of cutting which produces in *Nitella* a transitory "negative variation"[13]

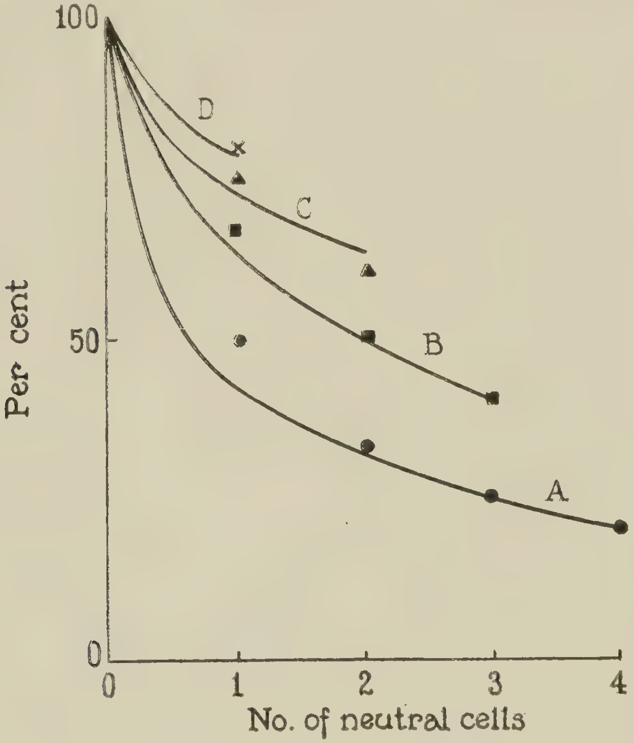


FIG. 3.

The curves show the calculated and the circles the observed P.D. for each group. Curve *A* for 1 positive cell shunted by 1, 2, 3, or 4 neutral cells; Curve *B* for a group of 2 positive cells shunted by 1, 2, or 3 neutral cells; Curve *C* for a group of 3 positive cells shunted by 1 or 2 neutral cells; Curve *D* for a group of 4 positive cells shunted by 1 neutral cell.

---

[10] The prediction is that the shunting effect will reduce the P.D. from 50 to 30, *i. e.*, to 60% of the original; in this case we start with 46 and 60% of this is 27.6 which is taken as 28.

[11] Average of 17 groups of 4.

[12] *i. e.*, that the reduction will be to 80% of 48 or 38.4.

[13] Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1928-29, xii, 167, 355.

the time curve of which somewhat resembles the current of action of muscle and nerve. If we arrange a group of *Nitella* cells with 0.001 M KCl at both ends and cut only 1 cell we obtain a much smaller P.D. than as if no shunting cells were present. On the other hand, if we cut all of them simultaneously we obtain about the same P.D. as if we cut 1 by itself. This is in accordance with expectation but no attempt was made to arrive at exact quantitative relations because the negative variation produced by cutting is so variable.

Experiments with Muscle.[14]

Since the fibres cannot be separated for experimental purposes no quantitative work was attempted. A normal muscle, short-circuited by a dead muscle (touching the leads) gives, of course, less current of action than when there is no shunt. The results of cutting the sartorius muscle of the frog are very irregular but when a sufficient number of experiments are averaged there is a rough proportionality between the number of fibres cut and the observed current of injury. If the muscle is completely cut across and a median longitudinal cut is made which partially or completely isolates the 2 halves it is found that each half gives approximately the same current of injury as was observed before the longitudinal cut was made. This is, of course, in accordance with expectation.

Other things being equal it would follow that if all the fibres are cut (or stimulated) a muscle or nerve with a small number of fibres should give as much current of injury (or current of action) as one with a greater number.

Summary: That the fibres of muscle or nerve, despite their parallel arrangement, act electrically as though in series may be explained by assuming that the inactive or uncut fibres act as shunts to those which are stimulated or cut. Experiments on *Nitella* support this assumption.

---

[14] We employed the sartorius or gastrocnemius of the frog (*R. catesbiana* and *R. pipiens*), leading off with Ringer's solution.

4551

Improved Method of Preparing Bacillus Typhosus Toxic Substances Necessary for Local Skin Reactivity.

GREGORY SHWARTZMAN.

From the Laboratories of the Mount Sinai Hospital, New York City.

In previous communications[1, 2, 3, 4] a phenomenon of local skin reactivity to *Bacillus typhosus* culture filtrates was described. The reactivity was induced by the injection of the filtrates into the skin of a rabbit. If 24 hours later an intravenous injection of the same filtrate was given to the rabbit, there appeared an extremely severe hemorrhagic necrosis at the site of the previous injection. The factors determining the local skin reactivity were termed "skin preparatory factors" and those involved in the production of local hemorrhagic necrosis following the intravenous injection were called "reacting factors." There were found a number of features which considered together distinguished this phenomenon sharply from the known phenomena of bacterial hyper-susceptibility and from the Arthus phenomenon. The same phenomenon was later reproduced with a variety of pathogenic microorganisms.[5] The skin preparatory factors of *Bacillus typhosus* were inactivated only by autoclaving, were not affected by a pH range of 4.0 to 9.0 and were specifically neutralized by immune sera. The *Bacillus typhosus* skin preparatory and reacting factors were obtained in filtrates of 6 day old cultures in tryptic digest broth.[2] These filtrates undoubtedly contain a considerable amount of extraneous material (products of tryptic digest and bacterial cell autolysis).

For the purpose of other work, it was desirable to obtain filtrates containing a minimal amount of these extraneous substances. After trying out a number of procedures, this object was attained by the use of washings of bacterial growth on solid media. The following is the method employed:

Kolle flasks containing plain veal infusion agar of pH 7.4 were seeded each with 3-4 cc. of 20 hour old plain broth culture of *Bacillus typhosus* (strain T_L) diluted 1-4 with 0.9% NaCl solution. The dilution was made immediately before use. After 20-22 hours of incubation the growth of each flask was washed off with 14-16 cc.

---

[1] Shwartzman, Proc. Soc. Exp. Biol. and Med., 1928, xxv, 560.

[2] Shwartzman, *J. Exp. Med.*, 1928, xlviii, 247.

[3] Shwartzman, Proc. Soc. Exp. Biol. and Med., 1928, xxvi, 131.

[4] Shwartzman, *J. Exp. Med.*, 1929, xlix, 593.

[5] Shwartzman, Proc. Soc. Exp. Biol. and Med., 1928, xxvi, 207.

of 0.9% NaCl solution containing 0.4% phenol. The washings were then pooled, centrifuged within the following 1-2 hours and the clear supernatant fluid filtered through Berkefeld "V" candles shortly after centrifuging. The filtrates prepared in this manner will be referred to as T_L "agar washings" filtrates.

The following is a summary of results obtained with these toxic substances:

Forty rabbits each received 6 simultaneous injections in dilutions of 1:2, 1:8, 1:16, 1:24, 1:36, and 1:48. The readings of the primary effect of these injections were made 23-24 hours later. The dilution 1:2 produced well-marked erythema (4+) in 1 rabbits, moderate erythema (2+) in 3 rabbits, slight reddening (1+) in 7 rabbits, and doubtful reddening or no erythema in the remaining 29 rabbits. With the dilution 1:8, only 1 rabbit showed a moderate erythema (2+), and the remaining rabbits very slight erythema or no reactions whatsoever. None of the higher dilutions had any visible primary effect upon the skin. Twenty-four hours after the intradermal injections, the rabbits were divided into 5 groups of six, and 2 groups of 5. Each group received a single intravenous injection of the filtrate. The first group received 1.8 cc., the second group 1.4 cc., the third group 1.0 cc., the fourth group 0.5 cc., and the sixth group received 0.3 cc., respectively, per kilo of body weight. There was an irregular general toxic effect of the filtrate. From 1 to 4 rabbits of each group were killed in 1-5 hours by the intravenous injection. The skin reactions of the surviving 26 rabbits were read 5 hours after the intravenous injection. Three rabbits showed no reactions (resistant). One of these had received intravenously 0.8 cc. and two 0.3 cc. of the filtrate per kilo of body weight. The remaining 23 rabbits showed very severe and typical reactions. Although, as observed before,[2] fluctuations were noticed in the skin preparatory effect of diluted filtrate, nevertheless there seemed to be a distinct reciprocal relationship between the amounts of filtrate necessary to be injected intradermally and intravenously in order to elicit the phenomenon. Thus, even the highest dilution (1:48) was able to prepare the skin of some rabbits for severe hemorrhagic necrosis providing from 0.8-1.8 cc. per kilo was injected intravenously. As the intravenous doses decreased, well-pronounced reactions were elicited only in areas prepared with dilutions lower than 1:48, so that in the last group which received intravenously 0.3 cc. per kilo, only areas prepared with dilution 1:2, 1:8 and 1:16 showed severe reactions.

Essentially similar results were obtained with 2 other batches of identically prepared T_L "agar washings" filtrates.

Summary and Conclusions: It is possible to obtain highly potent skin preparatory and reacting factors in filtrates of washings of 20-22 hour old *Bacillus typhosus* cultures on plain agar. Under these conditions there is avoided much of the extraneous material present in tryptic digest broth previously employed, as well as much of the bacterial autolysis occurring in the older method. This would indicate that the skin preparatory and reacting factors are bacterial soluble substances, the production of which is independent of massive cell destruction.

The filtrates prepared in this manner produce negligible primary effect upon the skin (erythema, swelling), notwithstanding their high skin preparatory potency. This is in agreement with the view previously expressed[1, 2, 5] that the skin preparatory potency of a given filtrate bears no relation to its primary effect on the skin.

It is of considerable interest to note that there exists a distinct reciprocal quantitative relationship between the skin and intravenous doses. This observation merits further study.

4552

Nicotine Tolerance in the White Rat.

C. H. THIENES.

From the Department of Pharmacology, University of Oregon Medical School, Portland, Oregon.

The minimal effective dose (M.E.D.) of nicotine for white rats of both sexes, from 29 to 33 days old, was 0.3 mg. per kilo body weight. This dose was sufficient to cause slight but definite weakness of the hind legs. After determining the M.E.D., one-half of each of 3 litters were injected twice daily with 2 to 3 times the M.E.D., producing symptoms ranging from instability to convulsions and prostration. At intervals of 3 and 9 weeks following the first injections, after a rest day of no injections, all rats were tested for the M.E.D. Whereas the controls, 10 in number (injected twice daily with salt solution), responded each time to 0.3-0.4 mg. nicotine per kilo body weight, the test rats, 13 in number, showed a definite decrease in susceptibility, in that at the end of 3 weeks, the M.E.D. averaged 0.65 mg. nicotine, and at the end of 9 weeks, 0.85 mg. per kilo body weight. This was considered to be evidence of an acquired nicotine tolerance.

An interesting phenomenon of "acute tolerance" was also observed. That is, the second injection of the day caused much milder symptoms than the first injection. Although not yet well worked out, it seems that the optimal interval between injections for demonstrating this phenomenon is from 2 to 4 hours.

4553

The Complement Fixation Test in Yellow Fever.*

MARTIN FROBISHER, JR.
From the International Health Division, Rockefeller Foundation, New York.

Yellow fever offers opportunity for study of the immune reactions of a virus disease under circumstances which eliminate error due to collateral antigens. According to Schultz[1] collateral antigens have been a frequent source of error in previous studies of the complement fixation reaction in virus diseases. Material quite free from collateral antigens can be obtained in yellow fever as antigen for the complement fixation test.

Aragao[2] has reported unfavorably upon the complement fixation test in yellow fever with antigens prepared from phenolated tissues. On the supposition that the antigenic substances in tissues affected by virus diseases might be within the cells, Ciuca[3] has made use of a process described as septic maceration in order to liberate the cellular contents. He reports success in differentiating between the 3 principal types of foot and mouth disease by complement fixation with antigens prepared in this way. The method of preparing the antigen for our tests is based on a procedure followed by Hindle[4] in making vaccine from yellow fever tissues. He produced rupture of the cells by causing a sudden change in the osmotic pressure of the fluid in which the tissues were suspended.

In preparing antigen, pieces of liver and spleen were taken at autopsy from monkeys which had died from experimental yellow fever. This material was ground thoroughly with sterile sand and soaked over night in the refrigerator in half its weight of 9.0% sodium chloride solution. The mixture was then suddenly diluted to the physiological concentration of salt by the addition of distilled water. The suspension of tissue thus prepared was centrifuged and passed through Berkefeld "V" filters. The filtrate constituted the antigen. For purposes of control an antigen was similarly prepared from tissues of a normal monkey.

---

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

[1] Schultz, E. W., *J. Immunol.*, 1928, xv, 229.

[2] Aragao, H. de B., *Compt. Rend. Soc. Biol.*, 1928, xlix, 1341.

[3] Ciuca, A., *J. Hyg.*, 1929, xxviii, 22.

[4] Hindle, E., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1929, xxii, 405.

In performing the complement fixation tests a simple standard procedure was used, employing an anti-sheep hemolytic system and 0.5 cc. quantities of each reagent. The sera to be tested were used undiluted after 30 minutes inactivation at 56° C. Fixation was allowed to proceed for 30 minutes at 37° C.

The results of these tests are summarized in the table. The human yellow fever sera listed as "recovered" or "immune" had all been tested for their power to protect monkeys against yellow fever and found to prevent death after the injection of at least 10 lethal doses of virus. Four sera, which were from persons supposed to have had yellow fever but which had failed to protect monkeys, have not been listed as "recovered" or "immune", but are included with the presumably normal sera. One of these, however, gave a positive reaction.

Except in this instance, sera which had failed to protect monkeys also failed to give complement fixation. On the other hand, all sera which protected monkeys gave positive fixation except one, which had failed to prevent fever in a monkey but had prevented death.

In addition to the tests for which results are given in the table, 38 retests were made with 14 of the same series and in all but one instance the results were in agreement with those obtained in the first tests of the same specimens.

In over 100 other tests made prior to those reported in the table, various antigens were tried, namely, urine from monkeys dead of yellow fever, alcoholic and aqueous extracts of their organs, and sera drawn from monkeys at the first elevation of temperature due to yellow fever. The results were found to be less reliable than those obtained later with the saline antigen.

Our results were in agreement with those of Aragao[2] in that yellow fever sera obtained during the first day of fever did not give strong or frequent reactions and that the sera of yellow fever convalescents did not fix complement in the presence of syphilis antigens.

Although the antigens used were made from tissues of monkeys inoculated with yellow fever virus from West Africa, positive complement fixation reactions were obtained with human sera from

TABLE I.
Result of Complement Fixation Tests in Yellow Fever.

Sources of sera tested	Antigen Used.					
	Saline extract of liver and spleen of yellow fever monkeys.			Saline extract of liver and spleen of normal monkey.		
	Positive	Negative	% correct	Positive	Negative	% correct
Normal men and monkeys	3	38	93	1	28	97
Recovered and immune men and monkeys	18	5	78	3	9	75
Convalescents from other diseases[*]	1[†]	19	95	0	18	100

[*] Syphilis 14, dengue 4, poliomyelitis 2 (1 human, 1 monkey).
[†] Syphilis.

South America and with the sera of monkeys which had been inoculated with virus from Brazil.

The method described appears to offer a means of identifying at least a useful percentage of the convalescents from yellow fever in a community. As far as can be determined by the tests which could be made with the small number of sera available, the reaction seems to be specific. Further work needs to be done in the search for more sensitive antigens, and the usefulness of the improved test should be determined by numerous trials under field conditions.

4554

Hemoglobin Standards in Normal Men.

M. M. WINTROBE.

From the Department of Medicine, Tulane University School of Medicine, New Orleans.

In an earlier report on normal blood standards,[1] a Newcomer hemoglobinometer, restandardized on the basis of 10 hemoglobin determinations made by the Van Slyke oxygen capacity method, was employed. Further standardization of this Newcomer instrument has led to the conclusion that the original correction curve was incorrect.

Additional hemoglobin determinations by the Newcomer and Van

---

[1] Wintrobe, M. M., and Miller, M. W., *Arch. Int. Med.*, 1929, xliii, 96.

Slyke methods have been carried out on 22 different samples of blood. Of this number 19 determinations (Table I) which checked very closely on 4 different Van Slyke instruments have been selected and a correction curve plotted on the basis of the total of 29 determinations.

TABLE I.

Hemoglobin Estimations by Newcomer and Van Slyke Methods.

Blood number	Hemoglobin.		
	Newcomer		Van Slyke
	%	gm.	gm.
11	73.0	12.35	16.70
12	84.5	14.30	18.58
13	67.0	11.35	16.31
14	39.0	6.60	9.40
15	70.0	11.85	15.41
16	80.0	13.55	16.65
17	57.0	9.75	13.75
18	54.0	9.14	12.12
19	76.0	12.88	16.96
20	48.0	8.15	12.41
21	45.0	7.62	10.86
22	45.0	7.62	11.19
23	74.0	12.52	16.86
24	59.5	10.06	15.59
25	61.0	10.30	15.54
26	85.0	14.38	18.77
27	73.0	12.33	16.19
28	76.0	12.88	17.01
29	66.0	11.13	15.30

The method employed for the plotting of the correction curve is that recommended by Pearl.[2] The hemoglobin determinations on the 2 instruments showed a high degree of correlation, the correlation coefficient (r) being .8669 ± .0984. From the fact that the correlation ratio (n) for these 29 determinations is not significantlv different from the correlation coefficient ($n = .9349\ \zeta = n^2 - r^2 = .1163 \pm .0835$) it has been concluded that the correlation is linear. Correction curves have been calculated by the method of averages and the method of least squares. None of these has been found altogether satisfactory. A logarithmic curve of the formula $y = 6.25 + 1.038\ x + .0286 \log x$ has finally been selected as the best fitting curve. (See Figure 1.)

As the result of this recalibration, the author has come to the conclusion that the hemoglobin figures already reported for normal young men in the South[1] are too low. By the new calibration the published average of 15.85 gm. becomes 17.0 gm., with 73% of the determinations ranging between 15.5 and 18.5 gm. This figure agrees exactly with Williamson's[3] finding in 214 men 20 to 80 years of age and is close to his result of 16.8 gm. in 36 men 20 to 30 years of age. The average of 317 relatively reliable hemoglobin determinations made in different parts of the world becomes 16.23 gm.

---

[2] Pearl, R., ''Medical Biometry and Statistics,'' W. B. Saunders Co., Phila., 1927.

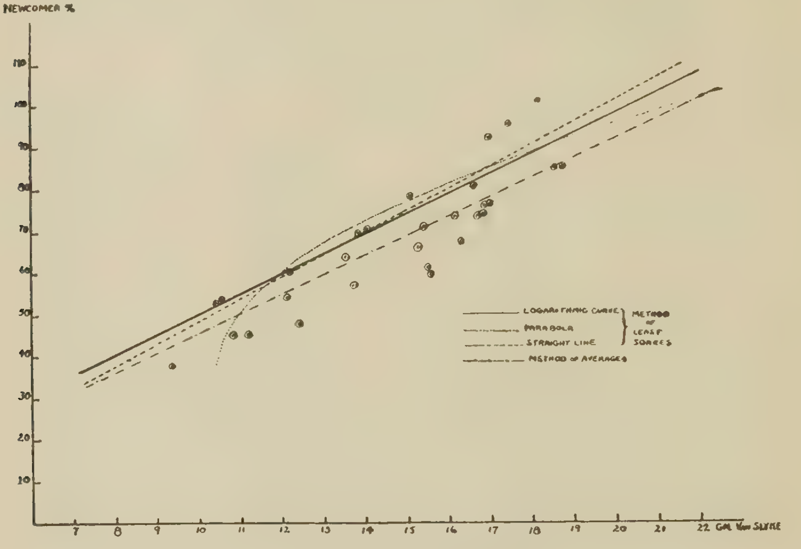


Fig. 1. Correction curves for Newcomer hemoglobinometer.

The average hemoglobin coefficient for the 100 men examined by the author becomes on the new scale 14.53 gm. instead of 13.66 gm., with 90% between 13 and 17.35 gm. The average hemoglobin coefficient based on 274 determinations made in different parts of the world is 14.61 gm. which agrees closely with the coefficient suggested by Osgood[4] (14.7).

Recalculation of color indexes on the basis of a hemoglobin coefficient of 14.6 gm. shows the following:

Haden[5] (Kansas City)	20	men,	averaging	1.07
Osgood[4] (Oregon)	137	”	”	1.00
Wintrobe and Miller[1] (Louisiana)	100	”	”	1.00
Gram and Norgäard[6] (Denmark)	7	”	”	0.96
Bie and Möller[7] (Denmark)	10	”	”	0.91

Total, 274 men, averaging 1.00.

---

[3] Williamson, C. S., *Arch. Int. Med.*, 1916, xviii, 505.

[4] Osgood, Edwin E., *Arch. Int. Med.*, 1926, xxxvii, 685.

[5] Haden, R. L., *Arch. Int. Med.*, 1923, xxxi, 765.

[6] Gram, H. C., and Norgäard, A., *Arch. Int. Med.*, 1923, xxxi, 164.

[7] Bie, V., and Möller, P., *Arch. d. mal. du coeur*, 1922, xv, 177.

Corresponding saturation indexes, based on a hemoglobin coefficient of 14.6 gm. and a volume coefficient of 41 cc. are:

Wintrobe and Miller (Louisiana)	100	men, averaging	1.02
Osgood (Oregon)	137	" "	0.98
Haden (Kansas City)	20	" "	0.96
Gram and Norgäard (Denmark)	7	" "	0.91
Bie and Möller (Denmark)	10	" "	0.89

Total, 231 men, averaging 0.99.

4555

Influence of Change of Sex on the Intensity of Heredity.

J. ARTHUR HARRIS, MARIAN A. WILDER AND BORGHILD GUNSTAD.

From the Department of Botany, University of Minnesota, Minneapolis, Minn.

Of recent years intense interest in the investigation of "sex limited" or "sex linked" inheritance by genetic experimentation has tended to divert attention from some of the phenomena of sex noted by statistical workers many years ago.

Pearson,[1] in studying Francis Galton's data for eye color in human ascendants and descendants, noted that the eye color of the younger generation is more highly correlated with an ascendant or collateral of the same than of the opposite sex, and suggests "that change of sex weakens the intensity of heredity." Lutz[2] determined the correlations between the eye color of the great grandparents and the great grandchildren and concluded that "every change of sex in the line of ancestry sensibly weakens the intensity of inheritance."

While the original data are given in multiple categories both Pearson and Lutz used the classical 4-fold table method of determining correlation, dividing the colors for both generations into 2 alternative classes at about the middle of the series of color categories. Since their work was done, papers on contingency[3] and on equivalent probability correlation[4] methods have appeared. It has, therefore, seemed worth while to recalculate these correlations by these 2 methods, and to compare the results with those obtained by Pearson and Lutz with the classical 4-fold method.

---

[1] Pearson, *Phil. Trans. Roy. Soc. Lond.*, 1900, A. 195, cxcv, 79.

[2] Lutz, *Biometrika*, 1903, ii, 237.

[3] Pearson, *Draper's Co. Res. Mem.*, Biom. Ser., 1904, i, 1.

[4] Pearson, K., *Draper's Co. Res. Mem.*, Biom. Ser., 1912, vii, 1.

The original data were recorded in the categories: 1, Light blue; 2, Blue, dark blue; 3, Grey, blue green; 4, Dark grey, hazel; 5, Light brown; 6, Brown; 7, Dark brown; 8, Very dark brown, black.

In determining the equivalent probability correlation coefficients the same groupings of the original 8 categories were made as were employed by Pearson and Lutz, that is, the series of classes was

TABLE I.

Correlations between eye color of ascendants and descendants in combinations involving various numbers of changes of sex.

Generation Number of Changes of Sex		Number of Cases	Correlation					
			4-Fold Method r	Average r	Equivalent Probability r_p	Average r_p	Contingency Coefficient C_1	Average C_1
Parental								
No change in sex	F-S	1000	.5503		.5285		.5888	
	M-D	1000	.5096	.5300	.4830	.5058	.4806	.5347
One change in sex	F-D	1000	.4370		.3677		.4842	
	M-S	1000	.4817	.4594	.4572	.4124	.4995	.4918
Grandparental								
No change in sex	GF-F-S	765	.4213		.3935		.5071	
	GM-M-D	739	.3180	.3696	.2825	.3380	.3634	.4352
One change in sex	GF-F-D	681	.3802		.3467		.4505	
	GF-M-D	687	.2969		.2570		.4066	
	GM-F-S	741	.2722		.2363		.3728	
	GM-M-S	756	.2523	.3004	.2130	.2632	.3882	.4045
Two changes in sex	GF-M-S	771	.3717		.3422		.3572	
	GM-F-D	717	.2205	.2961	.1777	.2600	.3712	.3642
Great Grandparental							8x8-fold	6x5-fold
No change in sex		368	.3471		.3569*		.4416	.3968
One change in sex		1113	.2217		.1894		.3716	.2942
Two changes in sex		1129	.1452		.1072		.3472	.2103
Three changes in sex		386	.0375		.0376*		.4370	.2979
Total material		2996	.1824		.1659		.2928	.2271

* Calculated by approximate method because the table for calculation of r_p from log X^2 and ${}_o\sigma_r$ does not extend beyond ${}_o\sigma_r = .08$.

broken into 2 groups at the line between "grey, blue green" and "dark grey, hazel."

In determining the contingency coefficients a certain amount of grouping was necessary to avoid the undue influence of cells of abnormally small frequencies. Pearson noted this source of error in the application of the contingency methods and it has been recently emphasized.[5] For the data given by Pearson the original 8x8-fold grouping has been maintained since N is generally large. For Lutz's data contingency coefficients were obtained from 8x8-fold tables and from tables with a 6-fold classification (classes 1-2, 3, 4, 5-6, 7, 8) for the offspring generation and with a 5-fold classification (classes 1-2, 3, 4, 5-6, 7-8) for the great-grandparental generation. Both sets of coefficients are given.

The results appear on Table I, which gives the individual coefficients as calculated by the 3 methods. In the case of the parental and grandparental generations coefficients are given for the individual combinations of GF = grandfather, F = father, S = son, GM = grandmother, M = mother, D = daughter. In the case of the great-grandparents' correlations the number of cases was not sufficiently large to make possible the calculation of the 16 possible coefficients representing each possible combination of sex. The coefficients for a combination of the data to show the magnitudes of the coefficients for 0, 1, 2 and 3 changes of sex are therefore substituted.

The first point of interest is the excellent agreement of the equivalent probability correlation coefficients with those calculated from the older 4-fold method. They substantiate fully the conclusions drawn by Karl Pearson and F. E. Lutz concerning the influence of change of sex on the coefficients measuring the resemblance of ascendants and descendants with respect to eye color.

The contingency coefficients are in general somewhat larger and somewhat more irregular. The only conspicuous exceptions to the conclusions to be drawn from the coefficients based on 4-fold groupings are those found in the case of 1 to 3 changes of sex in the great-grandparental correlation. Here the data are not merely less abundant but are probably less reliable. Because of the relatively small number of cases, the influence of cells with low frequencies is presumably large. This is evident from the fact that coefficients based on 8x8-fold tables are materially larger than those computed from 6x5-fold tables.

We have no suggestion as to the genetic interpretation of the results. The purpose of this note is merely to call attention again to the existence of relationships which seem to have been largely neglected of recent years, and which must be more fully investigated before we can feel that the problem of the inheritance of various human characteristics has been adequately treated.

---

[5] Harris and Treloar, *J. Am. Stat. Assn.*, 1927, xxii, 460.

4556

Reactions of Subcutaneous Tissue to Sodium Ricinoleate and Other Foreign Substances.

MARTHA W. EKOLA. (Introduced by W. P. Larson.)

From the Department of Zoology, University of Minnesota.

The purpose of this study was to ascertain whether there is a specific cytological response of subcutaneous tissue to detoxified toxin as compared with the reaction to a pure toxin. For further comparison of the reactions, one of the least toxic colloidal dyes was used.

Adult rabbits were injected subcutaneously with single doses of 1 cc. of 1% trypan blau in distilled water, 1 cc. of 1% sodium ricinoleate in distilled water, 1 cc. of diphtheria toxin in 1/500 dilution, and 1 cc. of diphtheria vaccine, *i. e.,* a combination of sodium ricinoleate and diphtheria toxin as prepared by Dr. W. P. Larson of the University of Minnesota. Subcutaneous tissue spreads were made by the spread method used by Maximow, von Möllendorf and others, and were fixed immediately in Zenker-formol. The stains used were Dominici's Eosin-Orange G and Toluidine Blue, Maximow's Hematoxylin Azure II-Eosin, Heidenhain's Iron Hematoxylin, and Weigert's Iron Hematoxylin. Tissue was taken at 45 minute, 1, 2, 3, and 4 day intervals for direct comparison of the effect of each of the named substances.

Very little difference in the appearance of the site of injection was noted in the 45 minute stage in the majority of cases. There were a few cases in which the area of injection turned greyish white when soap, toxin or vaccine was used, and then gradually became mildly inflamed. Some of the animals chewed at the irritated spot and produced an open sore, but no abscesses were found. When incision was made 45 minutes after injection, the subcutaneous tissue was very soft and gelatinous with a considerable quantity of fluid which probably was due in part to the undiffused irritant. The tissue was of pearly white color except with trypan blau, which had stained everything intensely blue.

In a few animals, 1 day after injection of each of the irritating substances, the area of injection became firm and thickened, and could not be raised from the muscular layers as could be done with the others. In such cases, the tissue was taken as near the site of injection as possible. The subcutaneous tissue was abundant, fairly gelatinous, and pearly white after injection of the soap, toxin, and vaccine, and blue after injection of trypan blau.

The 2 day stage was very similar to the one day stage with all the irritants used except with trypan blau, when the tissue was lighter blue.

The 3 and 4 day stages were very much alike in gross appearance, being somewhat thickened but not as firm as in the one day stage. The tissue in the trypan blau material was only faintly blue.

On microscopic examination the subcutaneous tissue from the trypan blau injected animals at the 45 minute stage showed, when it was dehydrated, cleared and mounted without further staining, that the cellular and fibrous elements had been diffusely colored by the vital dye as has been described by Downey. At this early stage the stained preparations did not show signs of inflammatory activity except for the presence of the dye particles in the cytoplasm of some of the fibroblasts and clasmatocytes. No polymorphonuclear leucocytes were seen.

In the corresponding stage in the soap material the general appearance was very similar, except that apparently empty vacuoles were present in the cells instead of dye particles.

Spreads made of toxin and vaccine injected subcutaneous tissues had more lymphocytes in some cases than did either the soap or trypan blau material, and the majority of these had very pycnotic nuclei. The cytoplasm of some of the larger lymphoid type of cells was very vacuolated, but contained no visible inclusions. There were definite signs of necrosis in the toxin injected tissue spreads.

With each of the irritants, polymorphonuclear leucocytes were most abundant one day after injection. The lymphocytes and polyblasts were fairly numerous in some spreads, with some variation between the individual animals.

On the second day there were great numbers of polyblasts, comparatively few typical fibroblasts and clasmatocytes, and fewer polymorphonuclear leucocytes than in the previous stage. The latter were breaking down and being phagocytozed by the polyblasts, clasmatocytes and fibroblasts. The soap, toxin and vaccine materials as well as the trypan blau injected subcutaneous tissue showed very little difference in reaction, a difference which did not surpass the variation in reaction of the individual animals to the same irritant.

On the third and fourth days the polyblasts were still the predominating cell type. There were but few typical clasmatocytes and fibroblasts present. The only marked difference with the substances used was the visibility of the ingested dye in the vacuoles of the cells in the trypan blau material, and the apparently empty vacuoles in the cells of the soap, vaccine and toxin materials.

These results would lead one to the conclusion that the soap and diphtheria-soap vaccine are not much more irritating to the subcutaneous tissue of rabbits than is trypan blau, which is considered the least toxic of the vital dyes. The diphtheria toxin alone, although no more specific in its effect on the cellular response, produces more necrosis than the other irritants used.

Missouri Branch.

4557

Studies of Experimental Streptococcus Arthritis. IV. Effect of Sodium Salicylate on Skin Allergy.

R. A. KINSELLA AND O. E. HAGEBUSH.

From the Departments of Internal Medicine and Bacteriology, St. Louis University School of Medicine.

The effect of sodium salicylate on the development of dermal allergy in the presence of hemolytic streptococcal arthritis was studied because this drug is commonly employed in the treatment of acute rheumatic fever, and because the studies of Swift[1] concerning the pathogenesis of this disease support the idea that allergy to streptococci of various kinds is a factor in its production.

As previously reported by us,[2] the production of arthritis in rabbits by the inoculation into a knee joint of 0.1 cc. of 24 hour broth culture of hemolytic streptococcus will be followed, in 6 days, by the appearance of a strongly positive local reaction to intradermal injection of a filtrate of a 5 day culture in Harley's medium. This skin reaction takes 12 to 24 hours to develop. All rabbits were tested previous to the experiment and found to give no such reaction.

In this study 24 rabbits were employed, 12 of which received sodium salicylate and 12 were used as controls. Of those which received sodium salicylate, 6 received the drug for a considerable period before receiving an intra-articular injection of 0.1 cc. of culture of hemolytic streptococcus; and 6 rabbits received the drug and the culture at the same time. The dose of sodium salicylate was 0.2 gm. per kilo in 4% solution and was given intravenously. The first series of 6 received 8 daily injections of salicylate and after an interval of 12 days, during which the animals were in good health, received 6 more injections given at 2 day intervals. After the injection of streptococci into the knee joint, 3 more daily injections of sodium salicylate were used and 3 days later skin tests were made. At this time 3 of the series were dead. The 3 survivors showed completely negative tests. Two of these animals were tested at intervals of a week and gave 2 more negative tests before dying; the other gave 3 more negative tests before dying. The animals receiving salicylate died more readily than the controls.

---

[1] Swift, H. F., Derick, C. L., and Hitchcock, C. H., *J. Am. Med. Assn.*, 1928, xc, 906.

[2] Kinsella, R. A., and Hagebush, O. E., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 642.

In the second series of 6 rabbits, the animals received the injection of salicylate 24 hours before the intra-articular injection was made. Thereafter 4 daily injections of salicylate were given and 4 animals survived. These were tested 8 days after arthritis was produced, and gave negative tests. Four further daily injections were made and 2 surviving rabbits gave negative tests.

Twelve control rabbits were used which gave strongly positive skin reactions 6 days after arthritis was produced, and the reactions remained positive. The arthritis was highly fatal to all the animals but more so to those animals receiving salicylate.

The conclusion is supported, that the intravenous injection of sodium salicylate prevents the development, in rabbits having a streptococcal arthritis, of a positive skin reaction to filtrate of hemolytic streptococcus culture.

4558

Nutritional Factors Affecting Growth of Rats on Diets Containing Sodium Benzoate.

WENDELL H. GRIFFITH.

From the Department of Biological Chemistry, St. Louis University School of Medicine.

In a recent report[1] it was shown that survival and growth of young rats on diets containing sodium benzoate were possible only if the diets furnished the nutrient materials necessary for growth and especially a supply of glycine, or of a precursor, adequate for the detoxication of the benzoate and for the formation of new tissue proteins. In connection with these experiments, it was noted that certain toxic benzoate diets were less toxic if the rats were kept in cages resting on shavings rather than in raised cages.[2] This fact suggested that glycine or some other dietary factor was obtained by the rats from the excreta or from the shavings. In these experiments the basal diet was supplemented daily with 150 mg. of dried brewer's yeast. Subsequent work showed that this quantity of yeast did not supply the optimum concentration of the vitamin B complex and also demonstrated that the ingestion of the excreta or shavings furnished the needed vitamins rather than glycine.

---

[1] Griffith, W. H., *J. Biol. Chem.*, 1929, lxxxii, 415.

[2] Griffith, W. H., *J. Biol. Chem.*, 1928, lxxviii, 24.

In the experiments reported in this paper, the basal diet was supplemented with 300 mg. of yeast. On this diet rats grew as well in raised cages as in cages on shavings. This was true in experiments in which the diet was fed *ad libitum* and also in experiments in which the food intake was restricted (Chart 1). Apparently on this diet the excreta or shavings furnished no additional *necessary* nutrient substances.

The effect on survival and growth of adding sodium benzoate to the new basal diet is also shown in Chart 1. The toxic concentration of benzoate in these experiments was 3.5%. The toxic concentration of benzoate in the previous experiments was 3%. The 2

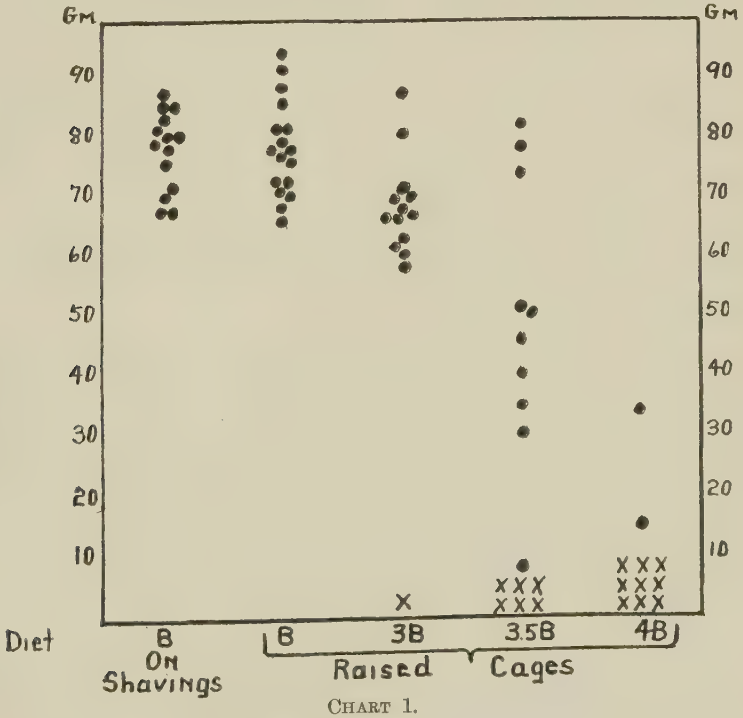


CHART 1.

The increase in weight of young male rats during a 40 day experimental period on the basal and benzoate diets. The number before the letter B shows the per cent of sodium benzoate in the diet. The food intake was restricted, each rat receiving the same quantity of food of equivalent calorific value. All rats except those in the first group were kept in individual raised cages. Deaths during the experimental period are shown by the letter x.

series differed only in the amount of the daily yeast supplement and it was concluded that the increased tolerance for benzoate, observed in the second series, was due partly to more efficient utilization of the food and partly to the presence of glycine in the additional yeast protein.

4559

Effect of Hormones of Anterior Pituitary on Thyroid Gland in the Guinea-Pig.

LEO LOEB AND R. B. BASSETT.

From the Department of Pathology, Washington University School of Medicine. St. Louis, Mo.

In former investigations Loeb[1, 3] and Loeb and Kaplan[2] have shown that feeding of tablets of anterior pituitary substance (Armour & Co.) prevents, in the guinea pig, compensatory hypertrophy which otherwise occurs in many cases after the greater part of the thyroid has been extirpated. H. A. McCordock[4] through feeding of these tablets prevented the marked cell proliferation which occurs in the guinea pig after administration of potassium iodide. There are also prevented the slight changes in colloid and in the size of the acinus cells caused by iodine. These experiments proved that in Armour's Anterior Pituitary preparation there is present a substance which prevents growth processes (hypertrophy and hyperplasia) in the thyroid of the guinea pig.

However, in larvae of amphibia, it has been shown that removal of the anterior pituitary may cause atrophic changes in the thyroid gland (B. M. Allen,[5] P. E. Smith[6]) or, conversely, that injection of acid extracts of anterior pituitary may stimulate the thyroid gland and cause morphological changes indicative of active secretion (E. Uhlenhuth and S. Schwartzbach[7]). These facts suggested to us the possibility that there may be present, also, in the mammalian anterior pituitary, substances which have a stimulating effect on the thyroid gland.

---

[1] Loeb, Leo, *J. Med. Res.*, 1920, xli, 481.

[2] Loeb, Leo, and Kaplan, E. E., *J. Med. Res.*, 1924, xliv, 557.

[3] Loeb, Leo, *Am. J. Pathol.*, 1929, v, 71.

[4] McCordock, H. A., *Am. J. Pathol.*, 1929, v, 171.

[5] Allen, B. M., *Anat. Rec.*, 1917, xi, 486.

[6] Smith, P. E., *Anat. Rec.*, 1917, xi, 57.

[7] Uhlenhuth, E., and Schwartzbach, S., *Brit. J. Exp. Biol.*, 1927, v, 1.

We prepared, from dried and powdered anterior pituitary of cattle, acid as well as alkaline extracts (subsequently neutralized) of which we injected daily 1 cc. intraperitoneally in each of a series of 19 guinea pigs, an additional animal as well as animals used in other series serving as controls. We used 2 different concentrations in both the alkaline and the acid extract. The injections extended over periods varying between 7 and 19 days. There resulted in every case hypertrophy of the thyroid gland; it varied in intensity in different animals but on the average was very pronounced and exceeded considerably the average obtained in cases of compensatory hypertrophy following extirpation of the thyroid gland. Especially in the younger animals the changes were very pronounced. All the signs of compensatory hypertrophy were thus imitated: Enlargement of the acinus cells; the appearance of granules in the portion of the cell directed towards the lumen of the acinus; softening and absorption of colloid; an irregular and often slit-like shape of the acini and, in certain cases, a great increase in mitoses. The hypertrophy exceeded considerably that found after administration of iodine in the guinea pig, where the mitotic proliferation is the principal effect obtained. Both acid and alkali extracts were able to produce this hypertrophy.

Of great interest is the relative or absolute loss in weight associated with the injections in the large majority of cases. It is especially marked in the heavy animals, while in the small (young) guinea pigs the majority retained approximately their weight or lost only a small amount. However, the relative loss of weight as indicated in the failure of the animals to gain during the period of injection is very great also in the young guinea pigs. In those animals which showed a distinct gain during the course of the experiments, the average intensity of hypertrophy is much less than in the others. This parallelism between intensity of hypertrophic changes and the weight curve of the guinea pigs suggests very strongly the conclusion that administration of the extracts caused an increased elimination of thyroid hormones into the circulation and thus caused an increase in metabolism. However, there remains the possibility that the extracts may affect the metabolism also directly and not merely by way of the thyroid.

We have, then, to deal with 2 opposite effects on the thyroid gland of substances obtained from the anterior pituitary. Feeding of Armour's tablets of anterior pituitary inhibits growth processes and function in the thyroid gland, whereas intraperitoneal injection of acid or alkaline extracts stimulates markedly the growth processes and apparently also the function of this organ. We have now a simple method which allows, in the guinea pig, the production of very intensive hypertrophy of the thyroid gland within a relatively short period of time.

Thus far these results apply only to the guinea pig, where the thyroid gland seems more responsive to stimulation than in certain other animals.

4560

Studies on Growth. II. Effect of Liver Extract on Food Consumption, Food Utilization and Growth.

WENDELL H. GRIFFITH AND CLAIRE E. GRAHAM.

From the Department of Biological Chemistry, St. Louis University School of Medicine.

In certain of our experiments it has been necessary to investigate the effect of the addition of liver to the diet of growing rats. Osborne and Mendel[1] have recently called attention to the marked acceleration of the growth rate which occurs under these circumstances. It was possible from our experiments to show the difference between the action of yeast and of liver on the consump-

TABLE I.
Effect of Liver Extract and of Yeast on Food Consumption, Food Utilization and Growth.

Diet	No. of rats*	Aver. increased wt., 40 day period	Aver. food intake	Calories per gm. increase in weight	Remarks
		gm.	cal.		
Mixed stock control diet	17	115	—	—	Diet fed *ad libitum.*
B + 0.3 gm. yeast	10	118	1345	11.4	” ” ” ”
B + 0.3 gm. yeast and liver extract	10	161	1630	10.1	” ” ” ”
B + 0.15 gm. yeast	17	62	975	15.7	Restricted food intake
B + 0.3 gm. yeast	16	80	975	12.2	” ” ”
B + 0.3 gm. yeast and liver extract	10	73	975	13.4	” ” ”

* Males, 30 days of age, were used.

---

[1] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1926, lxix, 661.

tion and the utilization of food. The addition of yeast to diets partially inadequate in the vitamin B complex increases the food intake of the rats but primarily increases the degree of utilization of the food ingested. The addition of liver to an adequate diet has little effect on the degree of utilization of the food but greatly increases the food intake. The effect of yeast and of liver on the consumption of food was demonstrated in experiments in which the diets were fed *ad libitum*. The effect of liver and of yeast on the utilization of food was demonstrated in experiments in which the food intake was restricted so that the rats received equal quantities of food of equivalent calorific value. The results of these experiments are shown in Table I. The extraordinary effect of the liver on the rate of growth of rats may be attributed to the increased food intake resulting from stimulation of the appetite. It is noteworthy that this stimulation of appetite was most evident during the first half of the 40 day period and was hardly apparent during the last half. This investigation of the relation of the appetite to food intake, food utilization, and growth is being continued.

4561

Pathogenic Properties of Organisms Obtained From Joints in Chronic Arthritis.

J. ALBERT KEY.

From the Shriner's Hospital and the Departments of Medicine and Surgery of Washington University, St. Louis, Mo.

During recent years considerable evidence has been brought forward to show that chronic arthritis is due to streptococci. Forkner, Shands and Paston[1] obtained *Streptococcus viridans* from the joints in 11 of 63 cases; Cecil, Nicholls, and Stainsby[2] obtained cultures of atypical streptococci from the blood in 49 of 78 cases.

I wish to report the results of cultures from the joints of 50 cases. In most instances the cultures were made from material obtained by aspiration of the joints and periarticular tissues. From 10 to 20 cultures were made from each joint and several varieties of culture media were used. The media which proved most successful were Rosenow's glucose brain broth and hormone medium, made from beef heart.

---

[1] Forkner, Shands and Poston, *Arch. Int. Med.*, 1928, xlii, 675.

[2] Cecil, Nicholls and Stainsby, *Arch. Int. Med.*, 1929, xliii, 571.

Eighty-eight aspirations were performed on 50 cases. Of these 39 were chronic atrophic arthritis, 6 were chronic hypertrophic arthritis, and 5 were periarthritis of the shoulder. In addition to the aspirations, biopsies were performed 9 times in 7 cases and cultures were made from small bits of synovial membrane and cartilage. Positive cultures were obtained in 20 of 39 cases (51%) of atrophic arthritis, in 3 of 6 cases (50%) of hypertrophic, and in 2 of 5 cases (40%) of periarthritis of the shoulder. The organisms obtained were staphylococcus, 15 times, minute bacilli in masses, 4 times, mixed cultures of the above organisms, 10 times. In 2 of the mixed cultures short chained non-hemolytic streptococci were associated with either diptheroids or staphylococci. There was no tendency for any one organism to be associated with either the atrophic or hypertrophic form of the disease.

The organisms were as a rule slow growing in the original cultures, and in some cases did not appear until 3 weeks after the inoculation. They did not grow in ordinary media and there was considerable difference in the amount of growth in the media used; while some of them died out within a few weeks. The cocci were uniformly gram positive, while the minute bacilli or diptheroids, were gram negative, but often contained polar bodies which were feebly gram positive. The pathogenic properties of the organisms were tested by injecting them into the knee joints of a series of rabbits 77 rabbits were injected with organisms obtained from 15 cases, and in each instance both knee joints were used, making a total of 154 experimental joints. In one of these rabbits were pyogenic joints produced. 40 of the joints were negative. In the remaining 114 there was a variable degree of chronic arthritis. These have been classified as +, ++, +++, and ++++, varying from slight excess of clear fluid with a small amount of synovial hyperplasia, to joints which were markedly distended with cloudy fluid and in which there was some periarticular thickening and marked hyperplasia of the synovial membrane; or joints in which there was relatively little excess fluid, but in which there was marked degeneration of the cartilage with considerable synovial proliferation and, in some cases, overgrowth of bone cartilage. The gross and microscopic changes in the joints and the cytology of the exudates were similar to those present in the chronic arthritic joints of humans; but there was no tendency for the disease to involve joints which had not been injected, and in none of these rabbits was the typical polyarthritis produced, although some of them were kept over a year.

When the animals were sacrificed small bits of the synovial membrane were planted in culture media. Of 90 joints, thus cultured, 25 were positive and 65 were negative. Nine of the positive cultures were contaminations, and in the remaining 16, organisms apparently identical with those injected were obtained.

We have thus produced the lesions characteristic of chronic arthritis in a series of rabbits by injecting organisms obtained from the joints of patients with the disease, and in a certain number of cases we have grown the organisms from the joints of the rabbits. We have injected these organisms into the circulation of rabbits with negative results. The staphylococci produced chronic arthritis in 66 of 84 instances (78%) and minute bacilli produced chronic arthritis in 34 of 50 instances (66%).

These results are open to 3 interpretations. (1) We are dealing with contaminations which are capable of producing chronic low grade arthritis, in animals. (2) We are dealing with non-specific organisms which are present in the tissues of patients with chronic arthritis, but which have no causative relationship to the disease. (3) Chronic arthritis is an infectious disease which may be due to any one of several organisms and is most often due to a low grade staphylococcus. It is probable that there is some as yet unknown abnormal susceptibility in those individuals who develop the disease.

4562

Oxidation of Lactic Acid by Dog Erythrocytes.

W. B. WENDEL. (Introduced by P. A. Shaffer.)

From the Laboratory of Biological Chemistry, Washington University School of Medicine.

The blood of certain species converts glucose to lactic acid but lacks the power of oxidizing either, and of oxygen consumption. The observation by Fürth and Lieben[1] of lactic acid destruction by horse erythrocytes was not confirmed by Warkany;[2] while Koechig[3] in this laboratory using the method described by Ray[4] failed to detect loss of lactate or formation of acetaldehyde. It appears that such "activation" of glucose as occurs in glycolysis does not alone suffice to accomplish the oxidation either of glucose or lactic acid—or an intermediate—by molecular oxygen. The same inference can be drawn from the similar behavior of cancer tissue or muscle extracts, the glycolyzing power of which is little affected by oxygen (Warburg and Meyerhof).

---

[1] Fürth and Lieben, *Biochem. Z.*, 1922, cxxviii, 144.

[2] Warkany, *Biochem. Z.*, 1927, clxxxiv, 480.

[3] Koechig, unpublished results.

[4] Ray, *Am. J. Physiol.*, 1927, lxxxii, 405.

Recently Barron and Harrop have shown that the addition of methylene blue to blood undergoing glycolysis causes a marked change in its behavior. There results an increase of O_2 consumption and of CO_2 production,[5] and a large part of the sugar which disappears fails to appear as lactic acid,[6] thus resembling reactions observed with muscle and other tissues. In the presence of the dye the blood respires with the oxidation, presumably, of either glucose or lactic acid or both. The rate of sugar disappearance is only slightly affected. The dye appears to supply an oxidation catalyst of a sort which is normally absent.

We have confirmed the observations of Barron and Harrop cited above and have attempted to decide whether it is the glucose which is oxidized and thus prevented from appearing as lactic acid, or whether lactic acid may be first formed and subsequently oxidized.

Our results show that dl-sodium lactate added to washed blood erythrocytes disappears on incubation in air in the presence, but not in the absence of methylene blue. The fact that O_2 consumption is increased indicates that the lactic acid is oxidized. It seems unlikely that it could be converted to some form of carbohydrate; no evidence for such possibility has been found. The action of methylene blue in bringing about the oxidation of lactate is not prevented by cyanide or fluoride. Since cyanide abolishes metal catalysis and fluoride prevents glycolysis, neither of these factors is necessarily concerned. In serum but under otherwise identical conditions neither lactate nor glucose is oxidized. The facts stated seem to indicate that corpuscles "activate" ("dehydrogenate") lactate, but not to a degree allowing oxidation by molecular oxygen or other components or catalysts (glutathione) present in blood. Methylene blue serves as a more efficient catalyst, presumably of a higher oxidation intensity, and perhaps resembling the iron-bearing catalysts which are supposed to participate in the more active oxidations in other tissues.

The red corpuscles of freshly drawn defibrinated dog blood were washed once with saline and suspended in an equal volume of an isotonic solution containing NaCl, sodium lactate and phosphate buffer (pH 7.3). One portion was precipitated immediately by the $ZnSO_4$ method of Somogyi.[7] Two other samples, one containing 0.005% methylene blue, were incubated in stoppered 300 cc. Erlenmeyer flasks. During incubation the flasks were rocked mechanically by motor to maintain uniform aeration. The short periods of incubation preclude errors from bacterial growth. Sugar ("true sugar") was determined on zinc filtrates, using a modified Shaffer-Hartmann reagent (unpublished No. 30 by Shaffer and Somogyi) which permits accurate determinations at very low sugar concentrations. Lactic acid was determined on copper-lime filtrates by the oxidation method.[8]

---

[5] Harrop and Barron, *J. Exp. Med.*, 1928, xlviii, 207.

[6] Barron and Harrop, *J. Biol. Chem.*, 1928, lxxix, 65.

TABLE I.

Description	Sugar in 100 cc. corpuscle suspension.		Lactic acid in 100 cc. corpuscle suspension.	
	Present mg.	Change mg.	Present mg.	Change mg.
Control (not incubated)	8	—	158	—
Control (incubated 3 hrs. 37° C.)	0	—8	167	+9
Incubated with methylene blue	5	—3	131	—27

The data given in the table illustrate the results obtained. Without methylene blue there is no evidence of loss of lactate, but rather in increase equal to the loss of glucose. In presence of the dye 20 to 35 mg. % of lactate disappears. Although the amount lost is not as great as with unwashed corpuscles the results are well beyond analytical errors and have been obtained in a series of such experiments.

4563

Anatomical Evidence for Existence of Enteric Reflex Arcs Following Degeneration of Extrinsic Nerves.

MYRON C. WADDELL. (Introduced by A. Kuntz.)

From the St. Louis University School of Medicine.

Dogs were subjected to an operation in which a large mesenteric artery and the nerves accompanying it were divided in order to

---

[7] Somogyi, M., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 353.

[8] Friedemann, Cotonio and Shaffer, *J. Biol. Chem.*, 1927, lxxiii, 335.

insure complete elimination of the extrinsic nerves to a segment of the intestine. The animals were kept alive from 15 to 60 days after the operation to allow ample time for degeneration of all extrinsic nerves. All of the material used in this study was prepared by the pyridine silver method.

Following degeneration of the extrinsic fibers, the intercellular plexus was greatly reduced, but a fine intercellular plexus in relation to the ganglion cells remained. The fibers which make up this plexus are of much smaller caliber than the extrinsic fibers which make up the greater part of the normal intercullular plexus. The large dendrites of the local ganglion cells which, according to Johnson[1] are the only processes remaining in the ganglia after degeneration of the extrinsic nerves were also observed. These are so much larger than the other fibers of the intercellular plexus that they cannot be confused with the latter.

An axon could be traced from its cell of origin to its termination on a neighboring cell in quite a number of cases. These fibers together with those which extend for some distance in either the myenteric or submucous plexus, or run from one of these plexuses into the other, make up the fine intercellular plexus which is present after degeneration of the extrinsic fibers.

A rich plexus composed of very fine varicose fibers lies directly beneath the intestinal epithelium. Fibers from this plexus ramify between the epithelial cells and form a network around their basal parts, where they terminate in fine terminal branches.[2] Fibers can be traced from this plexus to the submucous plexus. Degeneration of the extrinsic nerve fibers did not entirely destroy the fine subepithelial plexus. The fibers which remained are of the same caliber as those of the fine intercellular plexus. Fibers could be traced, after degeneration of the extrinsic fibers, to their termination in relation to both goblet cells and the other epithelial cells. The fibers supplying the goblet cells were interpreted as secretory; those supplying the other epithelial cells were interpreted as sensory or afferent. Since all extrinsic fibers had degenerated, it must be assumed that these fibers arise from cells in the enteric plexuses.

In view of the fact that degeneration of the extrinsic fibers destroys only a portion of the subepithelial plexus, leaving fiber terminations in relation to both secretory and other epithelial cells, and that synaptic relationships between enteric neurons have been demonstrated, the conclusion seems to be warranted that both afferent and efferent fibers are present and that reflex arcs exist within the wall of the intestine.

---

[1] Johnson, S. E., *J. Comp. Neur.*, 1925, xxxviii

[2] Hill, C. J., *Trans. Royal Soc. London,* 1927, ccxv,

4564

Influence of Serum From Pernicious Anemic Persons Treated by Liver on Growth of Seedlings.

LEON A. TSCHERKES. (Introduced by David I. Macht.)

From the Department of Morphology and Physiology, State Research Institute for General Pathology and Pharmaceutical Chemistry, Odessa.

The nature of the therapeutical effect produced by liver in cases of pernicious anemia is still under discussion, and we desired to establish whether the toxicity of blood of such patients undergoes any changes under treatment by liver, in order to determine the character of its influence on the blood.

We used the toxic blood of patients suffering from pernicious anemia, which impeded abruptly the growth of seedlings of *Lupinus albus* in the Shive nutritive solution, Macht.[1] We used the methods suggested by Macht, except that for the germination of *Lupinus albus* seedlings we used boiled sawdust instead of sphagnum moss.

We determined the phytotoxic index in 7 cases of pernicious anemia treated with liver during 7 to 12 months with excellent effect. Whereas before the treatment the patients all showed severe symptoms of typical pernicious anemia, after the above periods they were all entirely efficient, clinically healthy, and the picture of blood did not show any deviations from the norm. They did not stop taking liver, and yet we discovered in all cases very pronounced phytotoxicity of serum, such as Macht found in cases of untreated pernicious anemia studied by him, afterwards confirmed in our further experiments.

The phytotoxical index varied in 6 cases from 46 to 50%, and in one case was as high as 54%. The latter case showed a propensity to spontaneous remission even before the beginning of liver treatment. In 2 untreated cases the index was 46 and 47%, and in one case after a week's treatment it was 46%. Finally, in one case, after 7 weeks' treatment by liver the index went up from 46 to 51%. Besides this last mentioned case, we have at present none at our disposal in which the variations of index could be determined during the treatment, and we therefore refrain from coming to a conclusion regarding the possible diminution of phytotoxicity in the first period of treatment. Nevertheless, the fact that Macht[2] too observed some diminution of phytotoxicity of blood after a comparatively short liver treatment (2-4 weeks) deserves attention.

---

[1] Macht, *J. Pharmacol. and Exp. Therap.*, 1926, xxix, 461.

Thus, liver treatment, in its stereotyped form as applied at present, neither affects the phytotoxicity of blood, nor has any influence on *achylia* (Heath[3]) and *megalocythemia* (Etinger and Artinov[4]). These observations strengthen the grounds for denying the causative nature of liver treatment.

Why Upjohn, Isaacs and Gustafson[5] did not observe any abrupt increase of phytotoxicity of blood in cases of pernicious anemia remains unsolved. In our cases we (as well as Macht) not only found the average phytotoxical index much lower than those authors (48 instead of 76.6% of Upjohn and others), but also discovered that the amplitude of variations of phytotoxic coefficients, obtained from the same blood, with regard to all the seedlings used in the above experiment, had a different interval. In our cases this interval varied from 33 to 60%, whereas in the Upjohn, Isaacs and Gustafson experiments its amplitude varied from 59 to 94.3%.

The question of specificity of the above phenomenon must be answered in the negative, inasmuch as it has also been determined in cases of menstruation (Macht and Lubin[6]), and otosclerosis (Stern[7]), some psychoses (Herz and Weichbrodt[8]) (Looney and Macht[9]), and pemphigus (Macht and Pels[10]).

This phenomenon must be regarded only as a proof of the presence in the blood of substances highly toxic for vegetable protoplasm. The nature of these substances can evidently vary and they may be differentiated in other ways. This does not impair the usefulness in doubtful cases of the phytotoxic index for differential diagnosis, as in all the studied cases of diseases of hemopoietic system (Macht,[2] Tscherkes and Goldstein[11]), phytotoxicity does not exceed that of normal man. In our observations, the blood of patients suffering from cancer did not show higher toxicity than the blood of healthy individuals, and in this respect our data could not confirm Stern's observations.

---

[2] Macht, *J. Am. Med. Assn.*, 1927, lxxxix, 753.

[3] Heath, *J. Am. Med. Assn.*, 1928, xci, 928.

[4] Etinger and Artinov, *Vratckebnoye Dielo*, 1928, ix, 1408.

[5] Upjohn, Isaacs and Gustafson, *Arch. Int. Med.*, 1928, xlii, 909.

[6] Macht and Lubin, *J. Pharmacol. and Exp. Therap.*, 1924, xxii, 413.

[7] Stern, *Munch. Med. Woche*, 1926, lxxiii, 101.

[8] Herz and Weichbrodt, *Deut. Med. Woche*, 1924, l, 1210.

[9] Looney and Macht, *J. Biol. Chem.*, 1925, lxiii, Proc. lx.

[10] Macht and Pels, Proc. Soc. Exp. Biol. and Med., 1927, xxv, 237.

[11] Tscherkes and Goldstein, *Deut. Med. Woche*, 1929, lv, 434.

4565

Urethane—Rat Preparation for Study of Intestinal Peristalsis.

DAVID I. MACHT AND PAUL S. HILL, JR.

From the Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Baltimore, Md.

The following method of studying peristaltic movements of the intestines *in situ* has been found extremely useful by the writers in connection with an investigation of certain purgatives and is deemed worthy of description for the benefit of other investigators because it is simple, efficient and economical.

A white rat, previously starved for 24 hours, is anesthetized with an intraperitoneal injection of urethane. This anesthetic has been found by the writers to act with extraordinary rapidity in the rat, so that an injection of 1.25 gm. (using a 4% solution) per kilo weight of rat produces complete and deep anesthesia within 5 minutes, lasting for 5 or more hours without any appreciable depressant effect on the heart or respiration. Doses of 1.5 gm. per kilo produce an anesthesia lasting for 24 hours or more, while 1.0 gm. per kilo produces an anesthesia of shorter duration. The rat is tied on a small dissecting board and immersed in physiological saline solution at body temperature, care being taken to keep the head out of the water. A median incision is made through the abdominal wall, without producing any bleeding, and the intestines are allowed to float on the saline solution. For the study of the intestinal movements and the passage of food through the gastro-intestinal canal, a small quantity of India ink (0.5 of 1.0 cc.) is introduced directly into the stomach by injection through a fine catheter passed through the mouth and oesophagus into the stomach. The passage of food and gastric contents through the intestinal tract of the rat is a very rapid one, the whole length of the intestine being traversed ordinarily in an hour's time. This passage, or movement, of the intestinal contents mixed with the India ink or finely divided carbon particles can be plainly followed with the naked eye because the thin intestinal walls of the rat allow the dark contents to be seen through them. In this way, after the normal movements of the intestinal contents have been studied, the effects of various laxative and constipating substances can be readily investigated. Such drugs are mixed with the India ink and introduced into the stomach either by the "stomach tube" or by direct injection with a stout hypodermic needle into the stomach cavity. The action of powerful alkaloids, *e. g.*, pilocarpin, physostigmin, atropin, etc., can be exerted by injections into the subcutaneous or muscular tissue, while such drugs as act directly on muscle cells, *e. g.*, barium chloride, papaverin, benzyl alcohol, etc., may be applied directly to the outer intestinal walls. The use of India ink for visualizing the intestinal contents in these experiments has been suggested to the authors by the employment of India ink in mice by Loewe and Faure in another connection.[1]

4566

Effect of Liver Extract on Basal Metabolic Rate in Pernicious Anemia.

ADELAIDE BARER AND C. W. BALDRIDGE. (Introduced by Fred M. Smith.)

From the Department of Internal Medicine, State University of Iowa.

The following observations were made in an effort to determine whether or not the basal metabolic rate would be affected by the rapid increase in erythrocytes after the administration of liver extract.

We selected 2 female patients with uncomplicated pernicious anemia, neither of whom had had liver or liver extract. Each patient received 8 cc. of dilute hydrochloric acid daily. They were given general hospital diet for 36 days and high caloric general diet for 67 days, the total period of observation being 103 days. Liver extract was begun after a control period of 8 days.

The following determinations were made daily for 34 days, once during the sixth week and triweekly thereafter.

1. B.M.R.—Tissot spirometer and Haldane gas analysis. (Average of 2 analyses for each reading.)
2. Hemoglobin—Newcomer method.
3. Hematocrit—Van Allen method.
4. Reticulocyte counts—(1% brilliant cresyl blue, 500 cells being counted).

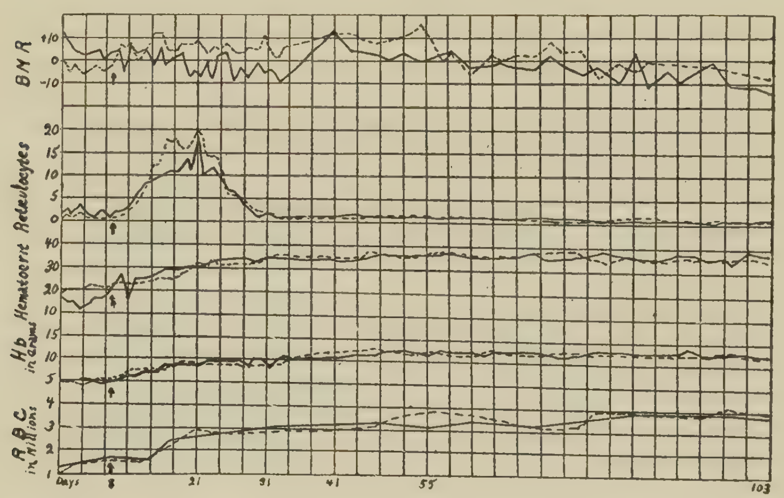
---

[1] Loewe, S., and Faure, G., *Arch. f. Exp. Path. u. Pharm.*, 1925, cvii, 271.

Erythrocyte counts were made about 4 times per week by different observers.

One patient received Armour's liver extract (1½ ounces daily), the other received Lilly's liver extract (3 vials daily).

The parallelism in the results was striking. The basal metabolic rate remained remarkably constant throughout the reticulocyte crises. In one patient the B.M.R. was found to be between +13 and +14% for 2 days at the beginning of the crisis, but all other readings were between +10% and —10% with no general tendency toward an elevation during the crisis.



The most marked increase in basal metabolic rate occurred on the fortieth day after a lapse of a week during which no estimations were made. One of the patients was found to have an increased basal metabolic rate again on the fifty-third day. We see no special significance in these occasional increases in metabolic rate.

A gradual fall in basal metabolism during the latter half of the period of observation was coincidental with a gain in weight. The calories per hour remained almost constant during this period.

Neither patient developed polycythemia.

Measurable serum bilirubin promptly disappeared after liver extract was begun.

The most marked increase in erythrocytes, hemoglobin, and cell volume occurred in a period of about 2 weeks after liver extract was begun. The erythrocytes increased from about 1 million to about 3 million in this period and other measurements increased accordingly.

Comments. 1. The *method used* did not reveal any significant change in the total body metabolic rate during reticulocyte crises. 2. The basal metabolic rate was practically unchanged during an increase in circulating erythrocytes from about 1,000,000 to about 4,000,000 per cu. mm.

4567

Experimental Hypochloremia.

GEORGE M. CURTIS AND G. A. PACHECO.

From the Department of Surgery, University of Chicago.

Hypochloremia is associated with a number of morbid conditions occurring spontaneously or produced experimentally. We have produced it by perfusing distilled water or other chloride free fluids through the peritoneal cavity of rabbits. Dialysis readily occurs through the peritoneal membrane.[1, 2, 3] When distilled water is injected intraperitoneally in rabbits a high percent of chlorides is rapidly deviated from the blood stream and the blood chloride falls.[4]

In 8 experiments distilled water at body temperature was perfused through the peritoneal cavity at the rate of 500 cc. per hour. Within an hour the respiratory rate increases and localized fibrillary twitchings appear. These become more generalized, and are followed by clonic and tonic convulsons of increasing severity. Spasmodic contractions of the diaphragm occur, and the animals die after 2 to 5 hours of perfusion. The blood chloride gradually falls, and a marked hypochloremia, as low as 118 mg. per 100 cc., results. The CO_2 combining power of the plasma falls. There is a moderate rise in the N. P. N., and a slight elevation of the urea. The secretion of urine soon diminishes and finally completely ceases. Hypoglycemia does not occur during the development of the symptoms and lethal effect. The addition of 0.12% glucose to the perfusion water in 7 other experiments had no notably beneficial influence.

The perfusion of Ringer's solution with glucose by the same methods resulted in no muscular twitchings or convulsions. These animals were alive and in good condition when the experiments were

---

[1] Curtis, G. M., *Biochem. Z.*, 1925, clxiii, 109.

[2] Heusser, H., and Werder, H., *Beit. z. Klin. Chir.*, 1927, cxli, 38.

[3] Engel, D., and Kerekes, A., *Zeit. f. d. ges. Exp. Med.*, 1927, lv, 574.

[4] Curtis, G. M., *Biochem. Z.*, 1927, clxxxvi, 95.

terminated at the end of 8 or 9 hours. The blood chloride was slightly elevated. There was a continuous secretion of urine, or even a diuresis.

When 0.9% pure sodium chloride, with or without glucose, was perfused, the animals were without symptoms and were readily kept alive for from 8 to 10 hours. During all experiments there was a continuous secretion of urine, even to a moderate diuresis. The blood chlorides were elevated, averaging 546 mg. per 100 cc. There was but little change in the CO_2 combining power of the plasma, and a slight decrease in the N.P.N. and urea. In one experiment the serum calcium fell to 2.9 mg. per 100 cc., with no change in the combining power of the plasma for CO_2. This animal showed no muscular twitchings or convulsions and was quiet during the latter part of the perfusion.

The perfusion of 4.2% glucose results in increased respirations, muscular twitchings and tremors, convulsions and eventually death in from 2½ to 7 hours. The terminal blood is hypochloremic, 136 mg. per 100 cc. There is an enormous rise in the blood sugar. Perfusion of 0.45% sodium chloride and 2.1% glucose had no such lethal effect and the animal was alive when the experiment was terminated at the end of 9½ hours. There was a slight rise in the blood chloride and an enormous increase in the sugar. Another animal was alive when the perfusion of an hypertonic solution, 0.9% sodium chloride and 2.1% glucose, was terminated at the end of 8 hours.

The perfusion of Ringer's solution with glucose, but *without sodium chloride,* resulted in increased respirations, muscular twitchings and convulsions, and the 2 animals died in 5 and 6 hours respectively. Hypochloremia developed, 181 mg. per 100 cc. The secretion of the urine diminished and then ceased.

These experiments led to the hypothesis that the loss of sodium chloride was an important factor in the development of the characteristic symptoms and the ordinarily fatal outcome following the perfusion of distilled water through the peritoneal cavity. The hypothesis was tested experimentally by supplying an adequate amount of sodium chloride to the blood stream throughout the perfusion with distilled water. This was accomplished by the timed intravenous injection of 2.5% pure sodium chloride by means of the Woodyatt pump. By this method it was possible to keep one animal alive 17½ hours, more than 5 times as long as after perfusion alone. Occasional mild muscular fibrillation occurred, but no severe tremors or no convulsions. A marked diuresis was observed. There was a slight fall in the whole blood chloride, however no hypochloremia as in the water experiments. The plasma chloride was high. There was no tetany although the blood calcium fell to 3.8 mg. per 100 cc.

When the combined intravenous infusion and transperitoneal perfusion was similarly maintained for 12 hours no symptoms developed. At the end of that time the intravenous injection of sodium chloride was stopped, but the perfusion of distilled water with glucose continued. Muscular twitchings then developed, became more severe and finally convulsions occurred and death followed after 5 hours of perfusion alone. In another experiment the simultaneous intravenous administration of Ringer's solution *without the sodium chloride* did not prevent the development of symptoms nor the lethal effect, and this animal died 5½ hours after the perfusion began.

These experiments represent a study of certain effects of peritoneal dialysis. It is difficult properly to evaluate, with the data at hand, the complex physico-chemical changes which result in the blood and tissues of the dialyzed animals. However, the experiments demonstrate the importance of sodium chloride to life and a state of well being.

4568

Phagocytosis of Red Blood-Cells Treated by Tannin.

JULES FREUND.

From the Henry Phipps Institute, University of Pennsylvania, Philadelphia.

Northrop and Freund[1] found that red blood-cells of sheep suspended in dextrose solution are agglutinated by electrolysis whenever the potential is depressed to 6 millivolts or less, except in the case of $MgCl_2$ and $CaCl_2$. When, however, cells are sensitized by specific antibody, ricin,[2] collodial stannic hydroxide,[3] an emulsion of paraffin oil,[1] they are agglutinated below about 12 millivolts, that is, at a potential higher than the critical potential for agglutination of unsensitized cells. This observation constitutes evidence that from the physical-chemical point of view specific antibody mediates agglutination by the same mechanism as ricin, collodial stannic hydroxide solution, and paraffin oil.

---

[1] Northrop, J. H., and Freund, J., *J. Gen. Physiol.*, 1924, vi, 603.

[2] Stillmark, H., *Arb. pharmacol. Inst. zu Dorpat*, 1889, iii, 59.

[3] Landsteiner, K., and Jagic, N., *Wien. Klin. Woch.*, 1904, xvii, 63.

Recently Reiner and Fischer[4] have been able to show that another substance, tannin (carefully neutralized), causes agglutination of red blood-cells of sheep, ox, and guinea pig and that red blood-cells treated with tannin become hemolyzed in the presence of guinea pig complement.

Repeating and extending Reiner's and Fischer's work I have found that erythrocytes of sheep exposed to 0.4 and 0.1 pro mille tannin solution, after they have been washed by centrifugalisation, are hemolyzed by the addition of guinea pig complement. If the mechanism of the action of tannin upon erythrocytes is similar to the action of immune serum, in which the electrical charge of the cells plays a rôle, agglutination and hemolysis should not occur when the suspending medium is free from electrolytes. When the experiment was repeated using isotonic dextrose instead of saline solution, neither agglutination nor lysis occurred. When tannin was added to red blood cells suspended in salt solution, the cells became agglutinated, but when the tubes were centrifugalized and the supernatant fluid replaced with dextrose solution, the cells could be suspended evenly and the addition of complement failed to cause lysis.

It was further observed that when from 0.4 to 0.1 pro mille tannin is added to red blood-cells suspended in either salt or dextrose solution a large number of the cells lose their original disc shape and become more or less angular. A similar change in shape takes place to a less extent when specific serum is added to red blood-cells. The final concentration of immune serum that caused such change ranged from 1:5 to 1:80.

Since immune serums prepare red cells for phagocytosis as well as for agglutination and lysis by complement, the question arises whether tannin prepares them for phagocytosis also. To test this possibility the following experiment was made. To 1 cc. of carefully neutralized tannin salt solution was added 0.2 cc. of a 1% suspension of washed erythrocytes of sheep. The mixture was kept at room temperature for 15 minutes and washed twice by centrifugalization. The red blood-cells were resuspended in 0.2 cc. of salt solution and mixed with 3 drops of a dense suspension of washed leucocytes of rabbit.

Both in the hanging drops and in the fixed preparations made from tubes containing red blood-cells treated with tannin and suspended in salt solution one could observe the following. A great number of red blood-cells were in clumps, some of which had surrounded leucocytes. Red cells were attached to many (often 60%) of the white cells, but only very exceptionally were they actually found within them. In preparations made from tubes containing red blood-cells treated with tannin and suspended in dextrose solution, or red blood-cells not treated with tannin and suspended in saline solution, neither agglutination nor attachment of red blood-cells to more than a very few leucocytes was found. Furthermore, in hanging drops from these tubes we could observe that moving red blood-cells would come into contact with leucocytes, slide on their periphery and then move away.

---

[4] Reiner, L., and Fischer, O., *Z. f. Immunitatsf.*, 1929, lxi, 317.

On completing this work I was of the opinion that the attachment of the red blood-cells to the leucocytes marked the beginning of phagocytosis, and that phagocytosis could be studied in the action of tannin upon erythrocytes. After the above paragraphs were written, a paper by Reiner and Kopp[5] appeared describing similar experiments (with guinea pig leucocytes) and arriving at similar conclusions, which they carried a step further. By using a longer period of incubation they observed complete phagocytosis of practically all the red blood-cells. This observation I have since confirmed.

4569

Correlation of Ultra-Violet Absorption to the Development of Immunity.

FREDERIC W. SCHLUTZ AND WILFRED W. WETZEL.

From the Institute of Child Welfare, the Department of Pediatrics, and the Department of Bio-Physics of the University of Minnesota.

It has been observed that the absorption spectrum for blood serum in the ultra-violet has a characteristic absorption band at 2800 Å. Judd Lewis[1] was the first to observe variations from the normal in the absorption curve of various pathological sera. In a second publication Lewis[2] shows that the protein content of serum is the absorbing constituent and gives curves for the absorption of the 3 protein fractions, pseudoglobulin, euglobulin, and albumin. Stenström and Reinhard[3] showed that a synthesis of the amino acids in the proportion in which they occur in blood serum gave a similar absorption curve to that of serum. They attribute the band chiefly to the effect of the tyrosine and tryptophane content of the serum. Studies have already been made of the protein content of pathological sera and the relation of globulin to albumin. Wells,[4] and Hurwitz and Meyer[5] found a variation of the globulin albumin ratio during the course of artificially stimulated immunity. They found that a temporary decrease of albumin with an increase of globulin took place during the development of immunity. Other investigators noted similar changes. The investigation of Lewis, Stenström, and others would seem to indicate that the ultra-violet spectroscope has the possibility of becoming a good instrument for determining whether immunity has or has not been acquired against certain infectious diseases.

---

[5] Reiner, L., and Kopp, H., *Z. f. Immunitatsf.*, 1929, lxi, 397.

[1] Lewis, S. Judd, *Proc. Royal Soc. London*, 1915, lxxxix, 327.

[2] Lewis, S. Judd, *Proc. Royal Soc. London*, 1922, xciii, 178.

[3] Stenström, W., and Reinhard, M. C., *J. Biol. Chem.*, 1925, lxvi, 819.

We propose a study of the absorption spectra of the blood serum in various contagious diseases common to childhood, covering the phases of normality, incubation period, height of the exanthem, period of convalescence and expected return to normal. By the correlation of data from a large number of cases, we hope to find some constant factor characteristic of immunity development.

Our investigations were carried out with a Hilger Type E-3 spectrograph and a Judd Lewis sector-photometer using an underwater tungsten spark.

So far we have collected data on 6 diphtheria immunization procedures with toxin antitoxin, the curve of the normal blood serum taken before immunization serving as control; 8 scarlet fever cases and 12 measles cases in various stages of the disease. In some cases it was possible to obtain the normal serum before onset of the disease to serve as a control. In most of the cases this was not possible. We do not feel that definite conclusions can be drawn from the data so far obtained, but a number of interesting changes have been observed.

A shift in wavelength of the absorption head and foot occurs which may prove to be of significance.

A somewhat constant increase of total proteins during the period in which immunity presumably develops, and the beginnings of a return to the normal after that period have been found.

In some of the cases, there is an indication of globulin albumin ratio increase.

From more spectrographic data, together with quantitative analysis, and fractionation of the sera, we can hope to show some consistent change in the proteins during the course of various diseases.

---

[4] Wells, H. G., *J. Immunol.*, 1924, ix, 291.

[5] Hurwitz, S. H., and Meyer, K. F., *J. Exp. Med.*, 1916, xxiv, 515.

We hope to correlate the spectrographic measurements of total protein changes and relative ratio changes of the protein fractions with the development of immune reactions in the course of disease.

4570

Decreased Resistance of Purified Bacteriophages to Disinfectants.

PAUL J. BEARD. (Introduced by E. W. Schultz.)

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

The surprising resistance of the bacteriophage to action of disinfectants has been commented upon by many observers. Thus d'Herelle[1] states that bacteriophage corpuscles survive 3 days' exposure to 0.5% mercuric chloride in physiological saline. Prausnitz and Firle[2] claim that resistance is above that of anthrax spores. Watanabe[3] noted well defined racial variations in the resistance of 'phages to phenol, 1% for 24 hours being sufficient to destroy some races, while 4% was necessary to inactivate others.

In order to determine whether the organic matter in the lysates exerts any protective action, 3 races of bacteriophage were purified according to the technique of Arnold and Weiss.[4] These purified 'phages gave negative Biuret, Xanthoproteic acid, Millon, Hopkins, Cole and Molisch tests. Non-purified 'phages were adjusted to the same lytic titer and the lysol resistance of both the purified and non-purified suspensions was compared. Tests of each 'phage were made in triplicate at various lysol concentrations. The results of these tests are summarized in the following protocol.

As is seen, the resistance of the 'phage to lysol was in every case markedly decreased. That the lysol solutions did not decrease greatly in activity is evidenced by the fact that after the 24 hour period a 3% concentration killed *B. subtilis* spores in 24 hours.

A decided decrease in the resistance to mercuric chloride was also noted, although accurate quantitative determinations have thus far not yet been made.

---

[1] d'Herelle, F., "The Bacteriophage and Its Behavior."

[2] Prausnitz, C., and Frile, E., *Centralbl. f. Bakt.*, Orig., 1924, xciii, 148.

[3] Watanabe, T., *Arch. f. Hyg.*, 1923, xcii, 1.

[4] Arnold, L., and Weiss, E., *J. Infect. Dis.*, 1925, xxxvii, 411.

TABLE I.

Comparison of Purified and Non-purified 'Phages to Lysol.

% lysol	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6
Normal coli phage	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +			
Pure coli phage	+ + +	+ + +	+ + +	+ + —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —
Normal protein phage	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	— — —	— — —	— — —	— — —	— — —	— — —	— — —
Pure protein phage	— + +	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —
Normal staph. phage	+ + +	+ + +	+ + +	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —
Pure staph. phage	+ + +	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —

+ = Lysin on subculture after 24 hours exposure.
— = Permanent inactivation.

It was further noted that these purified 'phage suspensions deteriorated rapidly at room temperature. Similar observations have been reported on enzymes stored in the absence of their substrates.

4571

Ovarian Hormone Content of Pregnant Cow's Urine.

C. W. NIBLER AND C. W. TURNER. (Introduced by Edgar Allen.)

From the Department of Dairy Husbandry, University of Missouri, Columbia, Mo.

The oestrous producing hormone has long been believed to be one of the chief causes of the development of the mammary glands during pregnancy. However, it was not until Fels[1] discovered the presence of the hormone in the blood during pregnancy, and further, that it was being excreted in large quantities in the urine during late pregnancy (Veler and Doisy[2]) was the fact established that the hormone is actually present in the blood and is being excreted in the urine. Recently Hisaw and Meyers[3] reported the presence of the hormone in the urine of a limited number of pregnant cows.

These fundamental observations are of great importance in the study of the causes of the development of the mammary gland. First, it is important to correlate the changes in the developing gland with the concentration of the hormone during various stages of pregnancy; and second, to determine the relation between the variation in the concentration of the hormone and the variation in the size of the gland and subsequent milk secretion. The purpose of the present communication is to present the results of a preliminary study having the above objectives.

The urine from 34 cows in various stages of gestation has been analyzed for its hormone content, using the Allen and Doisy[4] rat unit test. Doisy (unpublished work) has found that the hormone is absorbed by oil when mixed with urine in which it is present. In this series of tests, the oil was cleared in a centrifuge and injected. While this method of extraction may be considered only approximately quantitative, a rigid uniformity of procedure makes the results of comparative value.

The results are shown in Fig. 1. It will be noted that during the first one hundred days of pregnancy the daily excretion of the hormone is slight, averaging about 50 rat units. Following this period the average daily excretion begins to increase. This increase is at the rate of approximately 3.5 to 4 rat units per day until parturition. However, there was found considerable variation in the daily excretion of individual animals. This variation was found to be correlated to some extent with the previous yearly milk and butterfat production.

---

[1] Fels, E., *Klin. Woch.*, 1926, v, 2349.

[2] Veler, C. D., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 806.

[3] Hisaw, F. L., and Meyer, R. K., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 586.

[4] Allen, E., and Doisy, E. A., *J. Am. Med. Assn.*, 1923, lxxxi, 819.

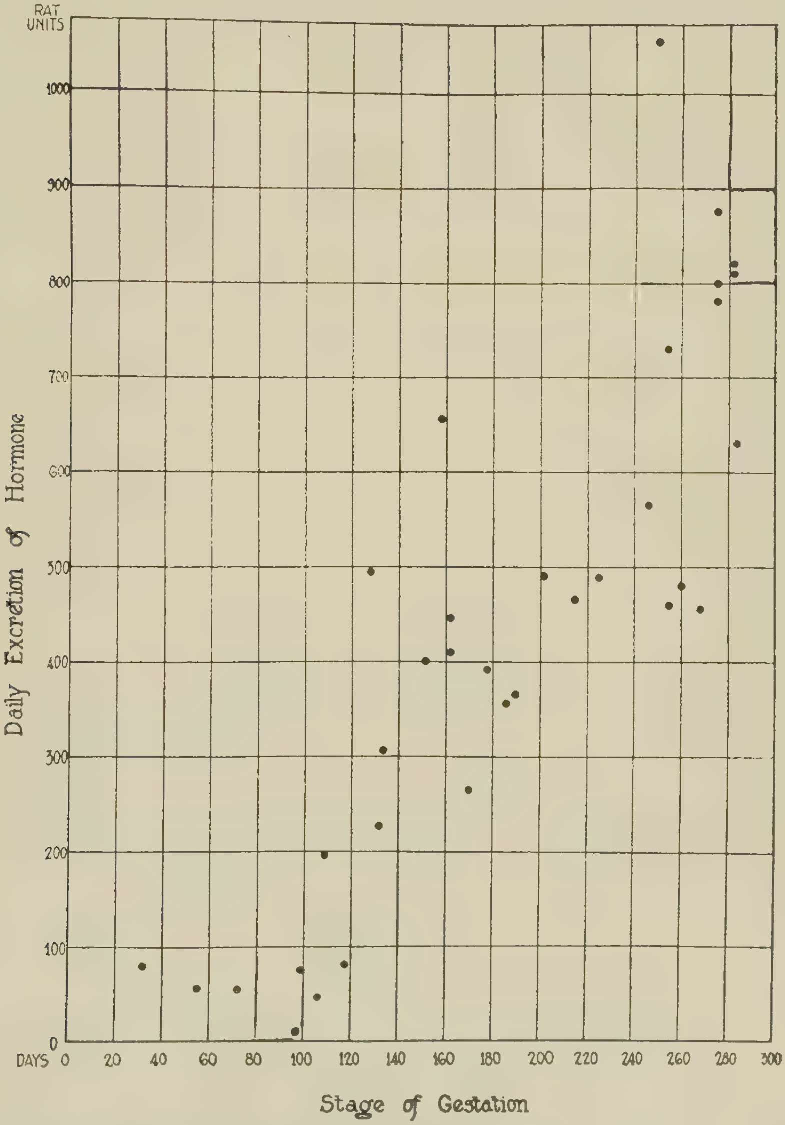


Fig. 1.

The variation in the average daily excretion of the ovarian hormone as extracted from cow's urine in different stages of gestation.

If the excretion of the hormone in the urine is a measure of its effective concentration in the blood during pregnancy, these observations may account for the rapid growth of the mammary gland during the latter stages of primigravida animals and also the variation in their subsequent milk and butterfat production.

---

[5] Loewe, S., *Klin. Woch.*, 1926, v, 576.

SECRETARY'S ANNUAL REPORT

to March 31, 1929

It is urged that members familiarize themselves with the Secretary's and Treasurer's reports, and thus reduce even further the delays and the inconveniences that arise from lack of information or misinformation as to the business or policies of the Society.

Your attention is particularly called to the

1. Financial Report.
2. Reduction in Dues.

FINANCIAL REPORT

There was a surplus for the year of $2,518.63. It will be recalled that last year there was also a surplus of $2,641.28.

REPORT ON PROCEEDINGS

The Editorial Board, anxious to maintain the standard of publication at a constantly higher level, has asked an increasing number of authors to radically revise their manuscripts or to withdraw trivial, duplicate, clinical, non-experimental, or grossly incomplete manuscripts. The response in practically every instance has been one of understanding and cooperation. The Board is encouraged to strive for ever higher standards of scholarship.

Sixty-four per cent of the manuscripts were preliminary; 36% complete. Average length of papers was 1.7 pages.

PROBLEMS

Application for Membership. It is generally agreed that the standards of the Society are primarily based upon the standards of the membership. In determining upon applications for membership, the vexing question arises when the applicant submits reprints in which he is coauthor only. Is he the assistant who carries out problems suggested to him, or is he the independent experimental research worker? In endorsing applications members are requested to give in such instances a full statement to the Membership Committee.

Promptness of Publication. To avoid needless delay in publication manuscripts should be in the office of the General Secretary by the first of the month. Manuscripts are printed in the order of acceptance. Secretaries of Branches are requested to aid in reducing delays.

Cost to Members. The Council has decided that members' dues be decreased from $5.50 to $4.00, that no charge be made for corrections, to take effect October 1, 1929.

MEMBERSHIP

Members, March 31, 1928	936	
Elected to March 31, 1929	66	
Honorary Members	8	
Total		1010
Resignations, year ending March 31, 1929	13	
Deaths	7	
Dropped for arrears	4	24
Net Membership		986

SUBSCRIPTIONS

Subscriptions, March 31, 1928	357	
Subscriptions, March 31, 1929		352
Free subscriptions, March 31, 1928	7	
Free subscriptions, March 31, 1929		7
Exchange subscriptions, March 31, 1928	8	
Exchange subscriptions, March 31, 1929		6
Total, March 31, 1929		365
Total No. of PROCEEDINGS distributed 3/31/28		1316
Total No. of PROCEEDINGS distributed 3/31/29		1351

OFFICERS

President: Peyton Rous.

Vice-President: David Marine.

Secretary-Treasurer: A. J. Goldforb.

Council: A. E. Cohn (1930), A. F. Hess (1930), W. W. Palmer (1931), H. D. Senior (1931), F. P. Gay (1932), G. B. Wallace (1932).

Past Presidents: F. B. Wilson, Simon Flexner, F. S. Lee, T. H. Morgan, James Ewing, Graham Lusk, W. J. Gies, Gary Calkins, G. B. Wallace, J. W. Jobling, S. R. Benedict.

Nominating Committee: John Auer, S. R. Benedict, R. Chambers, I. Greenwald, A. F. Hess, A. B. Luckhardt, V. C. Myers, P. A. Shaffer, D. D. Van Slyke.

Editorial Committee: Present members are A. J. Carlson, B. M. Duggar, M. H. Jacobs, John Kolmer, H. B. Lewis, W. deB. MacNider, A. M. Pappenheimer, A. J. Goldforb.

Membership Committee: Present members are E. A. Doisy, I. Greenwald, W. F. Petersen.

Financial Committee: Present members are William J. Gies, William H. Park, A. J. Goldforb.

PAST OFFICERS

Date	*President*	*Vice-Pres.*	*Secy.*	*Treas.*
1903-04	S. J. Meltzer	W. H. Park	W. J. Gies	G. N. Calkins
1904-05	S. J. Meltzer	J. Ewing	W. J. Gies	G. N. Calkins
1905-06	E. B. Wilson	E. K. Dunham	W. J. Gies	G. N. Calkins
1906-07	S. Flexner	E. K. Dunham	W. J. Gies	G. N. Calkins
1907-08	S. Flexner	T. H. Morgan	W. J. Gies	G. N. Calkins
1908-09	F. S. Lee	T. H. Morgan	W. J. Gies	G. Lusk
1909-10	F. S. Lee	W. J. Gies	E. L. Opie	G. Lusk
1910-11	T. H. Morgan	W. J. Gies	E. L. Opie	G. Lusk
1911-12	T. H. Morgan	P. A. Levene	G. B. Wallace	G. Lusk
1912-13	J. Ewing	P. A. Levene	G. B. Wallace	C. Norris
1913-14	J. Ewing	C. W. Field	H. C. Jackson	C. Norris
1914-15	G. Lusk	W. J. Gies	H. C. Jackson	J. R. Murlin
				Secy.-Treas.
1915-16	G. Lusk	G. N. Calkins		H. C. Jackson
1916-17	J. Loeb	W. J. Gies		H. C. Jackson
1917-18	W. J. Gies	J. Auer		H. C. Jackson
1918-19	W. J. Gies	J. Auer		H. C. Jackson
1919-20	G. N. Calkins	G. B. Wallace		H. C. Jackson
1920-21	G. N. Calkins	G. B. Wallace		H. C. Jackson
1921-22	G. B. Wallace	J. W. Jobling		H. C. Jackson
1922-23	G. B. Wallace	J. W. Jobling		H. C. Jackson
1923-24	H. C. Jackson	J. W. Jobling		V. C. Myers
1924-25	H. C. Jackson	J. W. Jobling		A. J. Goldforb
1925-26	J. W. Jobling	S. R. Benedict		A. J. Goldforb
1926-27	J. W. Jobling	S. R. Benedict		A. J. Goldforb
1927-28	S. R. Benedict	P. Rous		A. J. Goldforb
1928-29	S. R. Benedict	P. Rous		A. J. Goldforb

BRANCHES

Illinois.

Chairman: A. C. Ivy. Secretary: Lloyd Arnold. Members: 79.
Meetings: University of Illinois, October 30, 1928.
Billings Hospital, December 18, 1928.
Northwestern University Medical School, February 28, 1929.
Medical and Dental Arts Building, Chicago, May 15, 1929.

Iowa.

Chairman: H. A. Mattill. Secretary: H. M. Hines. Members: 25.
Meetings: University of Iowa, November 7, 1928.
University of Iowa, February 7, 1929.
University of Iowa, April 25, 1929.

Minnesota.

Chairman and Secretary: F. H. Scott. Members: 44.
Meetings: University of Minnesota Medical School, November 28, 1928.
University of Minnesota Medical School, February 27, 1929.
University of Minnesota Medical School, April 24, 1929.
University of Minnesota Medical School, May 29, 1929.

Missouri.

Chairman: John Auer. Secretary: P. A. Shaffer. Members: 40.
Meetings: St. Louis University School of Medicine, October 24, 1928.
Washington University School of Medicine, November 27, 1928.
St. Louis University School of Medicine, January 9, 1929.
Washington University School of Medicine, February 26, 1929.
St. Louis University School of Medicine, April 24, 1929.

New York.

Chairman: Peyton Rous. Secretary: A. J. Goldforb. Members: 626.
Meetings: New York Post-Graduate Hospital, October 17, 1928.
Rockefeller Institute, November 21, 1928.
College of Physicians and Surgeons, December 19, 1928.
Cornell University Medical College, January 16, 1929.
New York Academy of Medicine, February 20, 1929.
New York Academy of Medicine, March 20, 1929.
New York Academy of Medicine, April 17, 1929.
Yale University, May 25, 1929.

Pacific Coast.

Chairman: William Ophüls. Secretary: T. D. Beckwith. Members: 74.
Meetings: University of California, October 13, 1928.
Stanford University School of Medicine, December 12, 1928.
University of California School of Medicine, February 27, 1929.
Hopkins Marine Station, Pacific Grove, April 20, 1929.
University of California, June 20, 1929.

Peping (China).

Chairman: B. E. Read. Secretary: Hsien Wu. Members: 14.
Meetings: Peking Union Medical College, November 1, 1928.
Peking Union Medical College, January 24, 1929.
Peking Union Medical College, May 2, 1929.

Southern.

Chairman: John T. Halsey. Secretary: W. H. Harris. Members: 24.
Meetings: Tulane University, November 23, 1928.
Tulane University, March 21, 1929.
Tulane University, May 23, 1929.

Western New York.

Chairman: J. B. Sumner. Secretary: H. S. Liddell. Members: 40.
Meetings: Syracuse University, October 13, 1928.
Cornell University, December 15, 1928.
Clifton Springs Sanitarium, February 23, 1929.
University of Buffalo Medical School, April 17, 1929.

Wisconsin.

Secretary: Frederick L. Hisaw. Members: 20.

DEATHS OF MEMBERS

The Council records with regret the deaths of the following members: W. Denis, V. E. Emmel, Don R. Joseph, A. S. Loevenhart, John A. Mandel, A. A. Maximow, H. Noguchi, Thomas Osborne, H. M. Richards, G. E. Simpson, and Charles W. Young.

GIFTS

The Secretary wishes to acknowledge, with the thanks of the Council, the gift of back numbers from Thomas Flournoy, I. Greenwald, and W. F. Petersen.

TREASURER'S REPORT

April 1, 1928 to April 1, 1929

Receipts

Balance on hand, April 1, 1928		$ 3,867.59
Income, 1928-29:		
Dues	$ 5,270.93	
Reprints	1,996.85	
Excess space	1,732.25	
Authors' Changes, Bank Interest and Misc.	144.43	
Cuts	417.59	
Subscriptions	2,460.36	
Back Numbers Sold	429.25	
Total Annual Income		$12,451.66
Special Receipts—		
Interest on Endowment Fund		173.22
Deposit Account, Peking Union Medical College		200.00
Total Cash Available, April 1, 1928-April 1, 1929		$16,692.47

Disbursements

Publication Cost of PROCEEDINGS:		
Printing	$ 4,048.13	
Reprints	2,139.14	
Cuts	508.33	
		$ 6,695.60
Administration Expenses:		
Office Supplies and Postage	$ 980.44	
Salaries	2,085.00	
Storage and Insurance	69.75	
Miscellaneous	57.74	
Purchase of Back Numbers	4.50	
Overpaid	40.00	
		$ 3,237.43
Total Annual Disbursements		9,933.03
Transferred to Special Surplus Account		2,500.00
Reserve for Interest Due Endowment Fund		173.22
Cash Balance, April 1, 1929		4,086.22
Total		$16,692.47

Summary

Income (net)	$12,451.66	
Disbursements (net)	9,933.03	
Surplus for year		$ 2,518.63

Funds

Endowment Fund.

April 1, 1928	$10,479.22	
Contributions to April 1, 1929	144.50	
Interest to April 1, 1929	547.03	
Total		$11,170.75

Invested in N. Y. Title and Mortgage	$ 6,000.00	
Invested in R. R. Coop. Bldg. & Loan	4,997.53	
Interest due from General Fund	173.22	$11,170.75

Special Surplus Account

Invested in Title Guarantee and Trust Co.	$ 2,500.00

Life Membership Fund

Invested in R. R. Coop. Bldg. and Loan Assn.	$ 75.00

MEMO.
April 1, 1929.

Bills payable	None
Bills collectable	$ 1,259.90
Due from Life Membership Fund	3.71

Auditors' Report.

We have examined the accounts of the Society and the Treasurer's report, checked the book entries against the totals, and have verified the bank balance.

The auditors would again like to express the appreciation of the Society for the kindness of Mr. Alexander Dolowitz, C. P. A., who has once more examined the records and found them correct.

April 16, 1929.

Signed JOHN W. CHURCHMAN.
A. R. DOCHEZ.

MEMBERS' LIST (Alphabetical)

HONORARY MEMBERS

Councilman, William T.	Harvard University
Halliburton, W. D.	London, England
Lombard, Warren P.	University of Michigan
Porter, William	Harvard University
Reichert, E. T.	University of Pennsylvania
Richet, Charles	Paris, France
Schaffer, E. S.	Edinburgh, Scotland
Von Muller, Friedrich	Munich, Germany
Welch, W. H.	Johns Hopkins University

MEMBERS

Abbott, Alexander C.	University of Pennsylvania
Abel, John J.	Johns Hopkins University
Addis, Thomas	Medical School, Stanford University
Adler, Herman M.	Juvenile Psychopathic Institute, Chicago
Albus, William R.	American Type Culture Collection, Chicago
Alexander, Harry L.	Washington University
Allen, Bennet M.	University of California
Allen, Edgar	University of Missouri
Allen, William F.	University of Oregon
Alsberg, Carl L.	Stanford University
Alvarez, Walter C.	Mayo Clinic
Amberg, Samuel	Mayo Clinic
Amoss, Harold L.	Johns Hopkins Hospital
Anderson, E. G.	California Institute of Technology
Anderson, John F.	E. R. Squibb & Son
Anderson, Rudolph J.	Yale University
Andrews, Edmund	University of Illinois
Anson, Mortimer L.	Rockefeller Institute, Princeton, N. J.
Arnold, Lloyd	Loyola University
Asher, Leon	Berne, Switzerland
Ashman, Richard	Tulane University
Atchley, D. W.	Presbyterian Hospital, New York City
Atkinson, H. V.	Vermilion, S. D.
Atwell, Wayne J.	University of Buffalo, N. Y.
Aub, Joseph C.	Huntington Memorial Hospital, Boston
Auer, John	St. Louis University
Austin, J. Harold	University of Pennsylvania
Austin, W. C.	Loyola University
Avery, O. T.	Rockefeller Institute, N. Y. City

Bachem, Albert	University of Illinois Medical College
Baehr, George	Mt. Sinai Hospital, N. Y. City
Bagg, Halsey J.	Memorial Hospital, N. Y. City
Bailey, Cameron V.	N. Y. Post-Graduate Medical School
Baitsell, George A.	Yale University
Baldwin, W. Manning	Albany Medical College
Ball, G. H.	University of California, Los Angeles
Balls, A. K.	Bremen, Germany
Banta, A. M.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Banzhaf, Edwin J.	N. Y. Health Department
Barber, W. Howard	New York University Med. School

Barbour, Henry G.	University of Louisville
Bardeen, Charles R.	University of Wisconsin
Barlow, Orphus W.	Western Reserve University
Barnett, George D.	Stanford University
Barr, David P.	Washington University
Bass, Charles	Tulane University
Bast, T. H.	University of Wisconsin
Bauer, J. H.	Rockefeller Institute
Bauman, Louis	Presbyterian Hospital, New York City
Baumann, E. J.	Montefiore Hospital, N. Y. City
Baumberger, J. Percy	Stanford University
Bayne-Jones, S.	University of Rochester
Bazett, H. C.	University of Pennsylvania
Beard, H. H.	Western Reserve University
Becker, E. R.	Iowa State College
Becking, L. B.	Jacques-Loeb Lab., Pacific Grove, Calif.
Beckwith, T. D.	University of California
Behre, Jeannette A.	Cornell University Medical College
Belding, David L.	Boston University
Bell, E. T.	University of Minnesota
Benedict, S. R.	Cornell University Medical College, N. Y. City
Benton, Anne G.	Vassar College
Berg, B. N.	Columbia University
Berg, William N.	Berg Biological Laboratory, N. Y. City
Bergeim, Olaf	University of Illinois
Bergey, David H.	University of Pennsylvania
Berglund, Hilding	University of Minnesota
Bernhard, Adolph	Lenox Hill Hospital, N. Y. City
Beutner, R.	University of Louisville
Bills, C. E.	Mead, Johnson and Co., Evansville, Ind.
Binger, Carl A. L.	Rockefeller Institute, N. Y. City
Birkhaug, Konrad E.	University of Rochester
Bishop, George H.	Clayton, Mo.
Blackfan, K. D.	Harvard Medical School
Blake, F. G.	Yale University
Blakesley, Albert F.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Blatherwick, Norman R.	Metropolitan Life Insurance Co., New York City
Blau, Nathan F.	Cornell University Medical College
Blinks, L. R.	Rockefeller Institute
Bloom, William	University of Chicago
Bloomfield, A. L.	Stanford University Medical School
Bloor, W. R.	University of Rochester
Blum, Harold F.	Harvard Medical School
Blumgaart, H. L.	Beth Israel Hospital, Boston
Bodansky, A.	New York City
Bodine, J. H.	University of Pennsylvania
Bollman, Jesse L.	Mayo Clinic
Boothby, Walter M.	Kahler Hospital, Rochester, Minn.
Boots, Ralph H.	Rockefeller Hospital
Bowen, B. D.	Buffalo General Hospital
Boyd, Julian D.	State University of Iowa
Boyd, Theo. E.	Loyola University
Boyden, E. A.	University of Illinois
Bradley, H. C.	University of Wisconsin
Brand, Irwin	Montefiore Hospital, New York City
Branham, Sara E.	Hygienic Laboratory, Washington, D. C.
Brewer, Robert K.	Syracuse University
Briggs, A. P.	St. Louis University
Bronfenbrenner, J.	Washington University
Bronk, D. W.	Swarthmore College
Brooks, Clyde	University of Alabama
Brooks, Harlow	New York University
Brooks, Matilda M.	University of California

Brooks, S. C.	University of California
Broun, G. O.	St. Mary's Infirmary, St. Louis
Brown, E. D.	University of Minnesota
Brown, J. Howard	Johns Hopkins University
Brown, John B.	Ohio State University
Brown, L. A.	University of Iowa
Brown, Wade H.	Rockefeller Institute, N. Y. City
Buchanan, Robert E.	Iowa State College
Buckbinder, W. C.	Michael Reese Hospital, Chicago
Bulger, H. A.	Barnes Hospital, St. Louis, Mo.
Bull, C. G.	Johns Hopkins University
Bunting, C. H.	University of Wisconsin
Bunting, R. W.	University of Michigan
Burget, G. E.	University of Oregon Medical School
Burnett, Theodore C.	University of California
Burns, Robert K., Jr.	University of Rochester
Burr, Harold S.	Yale University
Burrows, M. T.	Pasadena, Calif.
Burton-Opitz, Russell	Palisade, N. J.
Byrne, Joseph	Fordham University
Calkins, Gary N.	Columbia University
Cannon, Paul R.	University of Chicago
Cannon, Walter B.	Harvard Medical School
Carlson, A. J.	University of Chicago
Carpenter, Charles M.	N. Y. State Veterinary College
Carter, Edward P.	Johns Hopkins Hospital
Cash, James R.	Peking Union Medical College, China
Castellani, Aldo	Tulane University
Cattell, McKeen	Cornell University Medical College
Caulfield, A. H.	University of Toronto
Cecil, R. L.	Cornell University Medical College
Chace, Arthur F.	N. Y. Post-Graduate Medical School
Chambers, Robert	New York University
Chambers, William H.	Cornell University Medical College
Cheer, S. N.	Peking Union Medical College, China
Chen, K. K.	Johns Hopkins University
Chidester, F. E.	University of West Virginia
Child, C. M.	University of Chicago
Chittenden, R. H.	Yale University
Chou, T.	Peking Union Medical College, China
Christian, Henry A.	Peter Bent Brigham Hospital
Christman, Adam A.	University of Michigan
Churchman, John W.	Cornell University Medical School
Clark, Guy W.	Lederle Lab., Pearl River, N. Y.
Clark, P. F.	University of Wisconsin
Clarke, Hans T.	Columbia University
Claussen, S. W.	Strong Memorial Hospital, Rochester, N. Y.
Clowes, G. H. A.	Eli Lilly and Co., Indianapolis, Indiana
Coca, A. F.	Cornell University Medical College
Coghill, G. E.	Wistar Institute
Cohen, Barnett	Johns Hopkins Medical School
Cohen, Martin	N. Y. Post-Graduate Medical School
Cohn, A. E.	Rockefeller Institute, N. Y. City
Cohn, Edwin J.	Harvard Medical School
Cohn, Isadore	New Orleans, La.
Cole, L. J.	University of Wisconsin
Cole, Rufus I.	Rockefeller Institute, N. Y. City
Cole, William H.	Rutgers University
Coleman, Warren	New York University Medical College
Collens, William S.	Brooklyn Jewish Hospital
Collett, Mary E.	Western Reserve University
Collier, William D.	St. Louis University

Collins, H. H.	University of Pittsburgh
Collins, Katherine R.	Spartansburg General Hospital, South Carolina
Collip, J. B.	McGill University
Conklin, E. G.	Princeton University
Cook, S. F.	University of California
Cooke, J. V.	Washington University
Coombs, Helen C.	New York University Medical School
Copenhaver, W. M.	Columbia University
Cori, Carl F.	State Institute of Malignant Diseases, Buffalo, N. Y.
Corley, Ralph C.	Tulane University
Corner, George W.	University of Rochester
Corper, H. J.	National Jewish Hospital, Denver, Colo.
Coulter, Calvin B.	Columbia University
Couret, M. J.	Tulane University
Cowan, John F.	Stanford University Medical School
Cowdry, E. V.	Washington University
Cowgill, George R.	Yale University
Crampton, C. Ward	N. Y. Post-Graduate Medical School
Crile, George W.	Western Reserve University
Crohn, Burrill B.	Mt. Sinai Hospital, N. Y. City
Crozier, W. J.	Harvard University
Cruickshank, E. W. H.	University of Patna, Patna, India
Csonka, F. A.	U. S. Bureau Chemistry, Washington, D. C.
Cullen, Glenn E.	Vanderbilt University
Cummins, Harold	Tulane University
Cunningham, R. S.	Vanderbilt University
Curtis, G. M.	University of Chicago
Curtis, Maynie R.	Columbia University
Cutler, Elliott C.	Western Reserve University
Dack, Gail M.	University of Chicago
Dakin, H. D.	Ossining, N. Y.
Danforth, Charles H.	Stanford University
Daniel, J. Frank	University of California
Daniels, Amy L.	University of Iowa
Daniels, Farrington	University of Wisconsin
Danzer, Charles S.	N. Y. Homeopathic Medical College
Davenport, C. B.	Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
Davies, H. Whitridge	University of Leeds, England
Davis, D. P.	University of Illinois
Davison, Wilburt C.	Duke University
Dawson, James A.	Harvard University
Day, A. A.	Northwestern University Medical School
De Eds, Floyd	Hygienic Lab., Washington, D. C.
DeGraff, A. C.	N. Y. University Medical School
Derick, C. L.	Peter Bent Brigham Hospital, Boston
Detwiler, S. R.	Columbia University
Deuel, Harry J., Jr.	University of Maryland Medical School
Dickson, E. C.	Stanford University Medical School
Dienes, Louis	Von Ruck Research Lab., Asheville, N. C.
Dieuaide, Francis R.	Peking Union Medical College, China
Doan, Charles A.	Rockefeller Institute
Dochez, A. R.	Presbyterian Hospital, N. Y. City
Dock, William	Stanford University
Doisy, Edward A.	St. Louis University
Donaldson, H. H.	Wistar Institute, Philadelphia
Donaldson, J. C.	University of Pittsburgh
Dooley, M. S.	Syracuse University
Drabkin, D. L.	University of Pennsylvania
Dragstedt, Carl A.	Northwestern University
Dragstedt, Lester R.	Northwestern University
Draper, George W.	Columbia University
Draper, John W.	New York City

Dresbach, M.	Albany Medical College
Dubin, Harry E.	Metz Laboratories, N. Y. City
DuBois, E. F.	Cornell University Medical College
Duggar, B. M.	University of Wisconsin
Dunn, Halbert L.	Baltimore, Md.
Dunn, L. C.	Columbia University
Dunn, Max	University of S. California
Dutcher, R. Adams	Pennsylvania State College
Duval, C. W.	Tulane University
Dye, Joseph A.	Cornell University Medical College

Eberson, Frederick	University of California Medical School
Ecker, E. E.	Western Reserve University
Eckles, C. H.	University of Minnesota
Eckstein, Henry C.	University of Michigan
Eddy, Walter H.	Columbia University
Edmunds, C. W.	University of Michigan
Edwards, D. J.	Cornell University Medical College
Eggston, Andrew A.	Manhattan Eye, Ear, Nose and Throat Hospital, N. Y. City
Eisberg, Harry B.	N. Y. University and Bellevue Medical College
Ellis, Max M.	Columbia, Mo.
Elsberg, Charles A.	Mt. Sinai Hospital, N. Y. City
Elser, W. J.	Cornell University Medical College
Engle, E. T.	Columbia University
Epstein, A. A.	Mt. Sinai Hospital, N. Y. City
Erdman, Rhoda	University of Berlin, Germany
Erlanger, Joseph	Washington University
Evans, Herbert M.	University of California
Ewing, James	Cornell University Medical College
Eyster, J. A. E.	University of Wisconsin

Faber, Harold K.	Stanford University Medical School
Fahr, George	University of Minnesota
Falk, I. S.	University of Chicago
Falk, K. George	Harriman Laboratories, N. Y. City
Falls, Frederick H.	University of Illinois
Famulener, L. W.	St. Luke's Hospital, N. Y. City
Farmer, Chester	Northwestern University
Faust, Ernest C.	Tulane University
Felton, L. D.	Harvard University
Ferry, R. M.	Harvard University
Field, Cyrus W.	New York City
Fine, M. S.	Battle Creek, Mich.
Fischer, Albert	Berlin, Germany
Fischer, Martin H.	University of Cincinnati
Fish, Pierre A.	Cornell University
Fishberg, Ella H.	New York City
Fisher, Nelson F.	St. Luke's Hospital, Chicago
Fitch, C. P.	University of Minnesota
Fitz, Reginald	Peter Bent Brigham Hospital
Fitzgerald, J. G.	University of Toronto
Fleisher, Moyer S.	St. Louis University
Flexner, Simon	Rockefeller Institute, N. Y. City
Flinn, Frederick B.	Columbia University
Florence, Laura	New York Homeopathic Medical School
Forbes, Alexander	Harvard University
Forbes, Henry	Milton, Mass.
Forkner, Claude E.	Rockefeller Institute
Fortuyn, A. B. D.	Peking Union Medical College
Foster, Goodwin L.	Columbia University
Foster, Nellis B.	New York Hospital, N. Y. City
Frank, Robert T.	Mt. Sinai Hospital
Fred, E. B.	University of Wisconsin

Freedman, Louis	Metz Laboratory
Freund, Jules	Henry Phipps Institute, Philadelphia
Fridericia, L. S.	University of Copenhagen, Denmark
Frobisher, Martin, Jr.	Rockefeller Institute
Fry, Henry J.	New York University
Fulmer, Ellis I.	Iowa State College
Fulton, John F.	Oxford, England
Funk, Casimir	Brussels, Belgium
Furth, Jacob	Henry Phipps Institute, Philadelphia
Gaebler, O. H.	Henry Ford Hospital, Detroit
Gager, C. Stuart	Brooklyn Botanic Garden, Brooklyn, N. Y.
Gahl, Rudolph	University of California
Gamble, James L.	Harvard University
Garbat, Abraham L.	Lenox Hill Hospital, N. Y. City
Gardner, Leroy U.	Saranac Lab. for Study of Tuberculosis
Garrey, Walter E.	Vanderbilt University
Gaskell, John F.	Cambridge, England
Gasser, Herbert S.	Washington University
Gates, Frederick L.	Rockefeller Institute
Gay, F. P.	Columbia University
Geiling, E. M. K.	Johns Hopkins Medical School
Gerard, R. W.	University of Chicago
Gesell, Robert A.	University of Michigan
Gettler, A. O.	University and Bellevue Medical College
Geyelin, Henry R.	Columbia University
Gibson, R. B.	University of Iowa
Gies, William J.	Columbia University
Githens, T. S.	Mulford Company, Philadelphia, Pa.
Givens, Maurice H.	Northwestern Yeast Co., Chicago
Glaser, Otto	Amherst College
Gold, Harry	Cornell University Medical College
Goldberg, S. A.	Bronx Hospital, N. Y.
Goldblatt, Harry	Western Reserve University
Goldbloom, A. Allen	Beth Israel Hospital, N. Y. City
Goldforb, A. J.	College of the City of New York
Goldschmidt, Samuel	University of Pennsylvania
Gortner, R. A.	University of Minnesota
Gould, Harley N.	Tulane University
Graham, Evarts A.	Washington University
Graves, William W.	St. Louis University
Green, Robert G.	University of Minnesota
Greenberg, David N.	University of California
Greene, Carl H.	Mayo Clinic
Greenwald, Isidor	Littauer Laboratory, N. Y. City
Greer, J. R.	University of Chicago
Gregory, Louise H.	Barnard College, Columbia University
Greisheimer, Esther M.	University of Minnesota
Griffith, Fred R., Jr.	University of Buffalo
Griffith, Wendell H.	St. Louis University
Gross, Erwin G.	Yale University
Gross, Louis	Mt. Sinai Hospital, N. Y. City
Gruber, Charles M.	Washington University
Guberlet, J. E.	University of Washington
Guenther, A. E.	University of Nebraska
Gurchot, Charles	Stanford University Medical School
Guthrie, C. C.	University of Pittsburgh
Guy, Ruth A.	New York City
Guyer, Michael F.	University of Wisconsin
Hadley, Philip	University of Michigan
Hafkesbring, H. Roberta	Tulane University
Hagan, William Arthur	Cornell University

Hale, Worth	Harvard Medical School
Hall, C. Ivan	University of Colorado
Halsey, John	Tulane University
Halsey, Robert H.	N. Y. Post-Graduate Medical School
Hamburger, W. W.	Rush Medical College
Hamilton, B. K.	Baltimore, Md.
Hammer, B. W.	Iowa State College
Hanke, Martin E.	University of Chicago
Hannon, R. R.	Rockefeller Institute
Hansman, G. H.	State University of Iowa
Hanson, Frank B.	Washington University
Hanzlik, P. J.	Stanford University Medical School
Hardesty, Irving	Tulane University
Harris, Isaac F.	Tuckahoe, N. Y.
Harris, J. Arthur	University of Minnesota
Harris, Reginald G.	Cold Spring Harbor, N. Y.
Harris, William H.	Tulane University
Harrison, R. G.	Yale University
Harrop, George A., Jr.	Johns Hopkins University
Harrow, Benjamin	College of the City of New York
Hartman, F. A.	University of Buffalo
Hartwell, John A.	Cornell University Medical College
Harvey, E. Newton	Princeton University
Harvey, Samuel C.	Yale University
Hastings, A. Baird	University of Chicago
Hastings, E. G.	University of Wisconsin
Hatcher, R. A.	Cornell University Medical College
Hathaway, Edward S.	Tulane University
Hawk, P. B.	New York City
Hayden, Charles E.	Cornell University
Haythorn, Samuel R.	Singer Research Lab., Pittsburgh, Pa.
Heft, Hattie L.	Columbia University
Heidelberger, Michael	Presbyterian Hospital, New York City
Heilbrunn, L. V.	University of Michigan
Helff, O. M.	State University of Iowa
Helmholz, Henry R.	Mayo Clinic
Hemmeter, John C.	Baltimore, Md.
Hench, Philip S.	Mayo Clinic
Hendrix, B. M.	University of Texas
Henrici, Arthur T.	University of Minnesota
Herrman, G. R.	Tulane University
Hertzman, A. B.	St. Louis University
Hess, Alfred F.	N. Y. University and Bellevue Medical School
Heymans, J. F.	U. de Gand, Belgium
Higgins, George M.	Mayo Clinic
Hill, Eben C.	Johns Hopkins University
Hill, Samuel E.	Rockefeller Institute
Hiller, Friedrich	University of Chicago
Himwich, H. E.	Yale University
Hines, H. M.	State University of Iowa
Hinrichs, Marie Agnes	University of Chicago
Hinshaw, H. C.	American University of Beirut, Syria
Hirschfelder, Arthur	University of Minnesota
Hisaw, F. L.	University of Wisconsin
Hitchcock, Fred A.	Ohio State University
Hoffman, George L.	Allegheny County Hospital, Pittsburgh, Pa.
Holm, George E.	Department of Agriculture, Washington, D. C.
Holman, Emil	Stanford University
Holman, W. L.	University of Toronto
Holmes, S. J.	University of California
Holt, L. Emmett, Jr.	Johns Hopkins University
Hooker, Davenport	University of Pittsburgh
Hooker, Sanford B.	Boston University
Hooper, Charles W.	Metz Laboratories

Hopkins, J. Gardner	Columbia University
Horsley, J. Shelton	St. Elizabeth's Hospital, Richmond, Va.
Hoskins, R. G.	Harvard Medical School
Howard, Harvey J.	Washington University
Howe, Paul E.	Department of Agriculture, Washington, D. C.
Howell, K. M.	Michael Reese Hospital
Howell, William H.	Johns Hopkins University
Howland, Ruth B.	New York University
Hubbard, Roger S.	Clifton Springs Sanitarium, N. Y.
Huber, G. Carl	University of Michigan
Huestis, R. R.	University of Oregon
Hunt, Reid	Harvard University
Hunter, Andrew	University of Toronto
Huntoon, F. M.	Mulford Co., Glenolden, Pa.
Hurwitz, Samuel	University of California
Hussey, Raymond	Yale University

Irving, Lawrence	University of Toronto
Irwin, Marion	Rockefeller Institute
Isaacs, Raphael	Simpson Memorial Institute, Ann Arbor
Ivy, Andrew	Northwestern University

Jackson, C. M.	University of Minnesota
Jackson, D. E.	University of Cincinnati
Jackson, Henry, Jr.	Boston City Hospital
Jacobs, M. H.	University of Pennsylvania
Jacobs, Walter A.	Rockefeller Institute, N. Y. City
Jacobsen, Walter C.	Albany Medical College
Jaffe, Henry L.	Hospital for Joint Diseases, N. Y. City
Jaffe, Richard H.	University of Illinois
Jeans, Philip C.	University of Iowa
Jennings, H. S.	Johns Hopkins University
Jobling, J. W.	Columbia University
Johns, Foster M.	Tulane University
Johnson, C. A.	University of Chicago
Johnson, T. B.	Yale University
Jonas, Leon	University of Pennsylvania
Jones, Frederick S.	Rockefeller Institute, Princeton, N. J.
Jordan, Edwin	University of Chicago
Jordan, H. E.	University of Virginia
Jorstad, L. H.	Barnard Free Skin and Cancer Hospital, St. Louis, Mo.
Joslin, E. P.	Boston, Mass.
Jungeblut, Claus	Stanford University

Kahn, Morris H.	Beth Israel Hospital, N. Y. City
Kahn, Morton C.	Cornell Medical College, N. Y. City
Kahn, R. L.	University Hospital, Ann Arbor, Mich.
Karsner, H. T.	Western Reserve University
Kast, Ludwig	N. Y. Post-Graduate Medical School
Katayama, I.	N. Y. Post-Graduate Medical School
Katz, L. N.	Western Reserve University
Keefer, Chester S.	Peking Union Medical College, China
Keeton, Robert W.	University of Illinois
Keitt, G. W.	University of Wisconsin
Kellogg, W. H.	University of California
Kendall, Arthur I.	Northwestern University Medical School
Kendall, E. C.	Mayo Clinic, Minn.
Kessel, John F.	University of California
Key, John A.	Shriner Hospital, St. Louis, Mo.
Killian, J. A.	N. Y. Post-Graduate Medical School
King, Helen D.	Wistar Institute, Philadelphia
Kinsella, Ralph A.	St. Louis University
Kirkbride, Mary B.	N. Y. State Dept. of Health, Albany, N. Y.

Kleiner, I. S.	N. Y. Homeopathic Medical School
Kleitman, Nathaniel	University of Chicago
Kligler, I. J.	Hebrew University, Palestine
Kline, B. S.	Mt. Sinai Hospital, Cleveland, Ohio
Klotz, Oskar	University of Toronto
Knowlton, Frank P.	Syracuse University
Knudson, Arthur	Albany Medical College
Koch, Elizabeth	University of Illinois
Koch, Fred C.	University of Chicago
Koch, Mathilda L.	Greenwich, Conn.
Kofoid, Charles A.	University of California
Kohn, Jerome L.	Mt. Sinai Hospital, New York City
Kolmer, John A.	University of Pennsylvania
Kopeloff, Nicholas	Psychiatric Institute, N. Y. City
Koppányi, Theodore	Syracuse University
Korns, John H.	Olean, N. Y.
Koser, Stewart	University of Illinois
Kramer, Benjamin	Brooklyn Jewish Hospital
Krasnow, Frances	Columbia University
Krumbhaar, E. B.	University of Pennsylvania
Krumwiede, Charles	N. Y. City Dept. of Health
Kruse, Theophile K.	University of Pittsburgh
Kugelmass, I. Newton	New York City
Kunde, Margaret M.	University of Chicago
Kuntz, Albert	St. Louis University

Ladd, William S.	Presbyterian Hospital
Lamar, R. V.	University of Georgia
Lambert, R. A.	Rockefeller Foundation
Lamson, Paul D.	Vanderbilt University
Lamson, Robert W.	Los Angeles, Calif.
Lancefield, D. E.	Columbia University
Landsteiner, Karl	Rockefeller Institute, N. Y. City
Langstroth, Lovell	San Francisco, California
Larsell, Olaf	University of Oregon
Larson, Edward	University of Alabama
Larson, J. A.	Johns Hopkins Hospital
Larson, W. P.	University of Minnesota
Lashley, K. S.	Institute for Juvenile Research, Chicago
Lathrop, Carl O.	University of Buffalo
Laughlin, H. H.	Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
Laurens, Henry	Tulane University
Leake, C. D.	University of California Medical School
Leake, J. P.	Hygienic Laboratory, Washington, D. C.
Lee, Ferdinand C.	Johns Hopkins University
Lee, Frederick S.	Columbia University
Lee, Milton O.	Harvard University
Leiter, Louis	University of Chicago
Leonard, C. S.	Hartsdale, N. Y.
Levene, P. A.	Rockefeller Institute, N. Y. City
Levene, Victor	Creighton University
Levin, Isaac	New York City
Levine, Max	Iowa State College
Levine, Michael	Montefiore Hospital, N. Y. City
Levine, Samuel Z.	Cornell University Med. College
Levinson, Samuel A.	University of Illinois
Levy, Robert L.	Presbyterian Hospital, N. Y. City
Lewis, Howard B.	University of Michigan
Lewis, Paul A.	Rockefeller Institute, Princeton, N. J.
Lewis, Robert C.	University of Colorado
Liddell, H. S.	Cornell University
Lieb, C. C.	Columbia University
Lillie, Frank R.	University of Chicago
Lillie, Ralph S.	University of Chicago

Lim, Robert K.	Peking Union Medical College, China
Lipmán, Charles B.	University of California
Little, C. C.	University of Michigan
Liu, Shih-hao	Rockefeller Institute
Locke, A. P.	St. Luke's Hospital, Chicago
Loeb, Leo	Washington University
Loeb, Robert F.	Presbyterian Hospital, N. Y. City
Loebel, Robert O.	Cornell University Med. College
Loehwing, W. F.	University of Iowa
Long, Perrin H.	Rockefeller Institute
Longcope, W. T.	Johns Hopkins University
Looney, J. M.	Jefferson Med. College
Lorenz, William F.	University of Wisconsin
Lucas, William P.	University of California
Luck, J. Murray	Stanford University
Lucke, Baldwin	University of Pennsylvania
Luckhardt, A. B.	University of Chicago
Lund, E. J.	University of Texas
Lundsgaard, Christen	Medical Clinic A, Copenhagen, Denmark
Lusk, Graham	Cornell University Medical College
Lyle, W. G.	New York City
Lyman, J. F.	Ohio State University
Lynch, Clara J.	Rockefeller Institute, N. Y. City
Lyon, E. P.	University of Minnesota
McCann, William S.	University of Rochester
McClendon, J. Francis	University of Minnesota
McClintock, John T.	University of Iowa
McCollum, E. V.	Johns Hopkins University
McCordock, H. A.	Washington University
McCrudden, Francis H.	Boston, Mass.
McCutcheon, Morton	University of Pennsylvania
McEllroy, W. S.	University of Pittsburgh
McGinty, Daniel A.	University of Michigan
McIver, M. A.	Mass. General Hospital
McJunkin, Frank A.	Loyola University
McKinley, E. B.	School of Tropical Medicine, San Juan, Porto Rico
McLean, Franklin C.	University of Chicago
McMaster, Philip D.	Rockefeller Institute, N. Y. City
McMeans, J. W.	Pittsburgh Hospital
McQuarrie, Irvine	University of Rochester
MacDougal, D. T.	Desert Laboratory, Tucson, Arizona
MacDowell, E. Carlton	Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
MacKay, E. M.	Rockefeller Institute
MacLeod, Grace	Columbia University
MacLeod, J. J. R.	Marischal College, Aberdeen, Scotland
MacNeal, Ward J.	N. Y. Post-Graduate Medical School
MacNider, William de B.	University of North Carolina
Macallum, A. B.	McGill University
Macht, David I.	Johns Hopkins University
Mackenzie, George M.	Mary Imogene Bennett Hosp., Cooperstown, N. Y.
Magath, T. B.	Mayo Clinic
Mallory, Frank	Boston City Hospital
Maltaner, Frank	N. Y. State Department of Health, Albany
Mandel, Arthur R.	N. Y. University and Bellevue Medical College
Mandel, John A.	N. Y. University and Bellevue Medical College
Mann, Frank C.	Mayo Clinic
Mann, Hubert	United States Veterans' Hospital, N. Y. City
Manville, Ira Albert	University of Oregon
Manwaring, W. H.	Stanford University Medical School
Marine, David	Montefiore Hospital, N. Y. City
Marriott, McKim	St. Louis Children's Hospital
Marshall, E. K., Jr.	Johns Hopkins University

Marshall, John A.	University of California
Marshall, Max S.	University of California
Martin, E. G.	Stanford University
Mason, K. E.	Vanderbilt University
Master, Arthur M.	New York City
Matas, Rudolph	New Orleans, La.
Mattill, Henry A.	State University of Iowa
Mavor, James A.	Union College
May, R. M.	Paris, France
Mayerson, H. S.	Tulane University
Maynard, L. A.	Cornell University
Medes, Grace	University Hospital, Minneapolis
Medlar, E. M.	Mt. McGregor, N. Y.
Meek, Walter J.	University of Wisconsin
Megrail, Emerson	Western Reserve University
Mehrtens, Henry G.	Stanford University Medical School
Meigs, E. B.	Dairy Division Exp. Station, Beltsville, Md.
Meleney, F. L.	Presbyterian Hospital
Meleney, Henry E.	Vanderbilt University
Melhus, I. E.	Iowa State College
Mellon, Ralph R.	Western Pennsylvania Hospital, Pittsburgh
Mendel, Lafayette B.	Yale University
Mendenhall, W. L.	Harvard University
Menne, Frank R.	University of Oregon
Menten, Maude L.	University of Pittsburgh
Meyer, Adolph	Johns Hopkins University
Meyer, A. L.	Johns Hopkins University
Meyer, A. W.	Stanford University Medical School
Meyer, K. F.	University of California
Michaelis, Leonor	Johns Hopkins University
Miles, W. R.	Stanford University
Miller, C. Philip, Jr.	University of Chicago
Miller, E. G., Jr.	Columbia University
Miller, G. H.	University of Iowa
Miller, Joseph L.	St. Luke's Hospital, Chicago
Millet, John A. P.	Stockbridge, Mass.
Mills, Clarence A.	Peking Union Medical College
Minot, George R.	Boston City Hospital
Mirsky, Alfred E.	Rockefeller Institute
Mitchell, O. W. H.	Syracuse University
Moise, T. S.	Yale University
Moore, A. R.	University of Oregon
Moore, Lillian M.	University of California
Moore, M. M.	University of Oregon
Morgan, T. H.	California Institute of Technology
Morse, Arthur	New Haven Hospital
Morse, Withrow	Jefferson Medical College
Mosenthal, H. O.	N. Y. Post-Graduate Medical School
Muckenfuss, R. S.	Rockefeller Institute
Mudd, Stuart	University of Pennsylvania
Mudge, C. S.	University of California
Muehlberger, C. W.	University of Wisconsin
Mueller, E. F.	Hamburg, Germany
Mueller, J. Howard	Harvard University
Mulinos, M. G.	Columbia University
Muller, G. L.	Boston City Hospital
Muller, Herman J.	University of Texas
Murlin, John R.	University of Rochester
Murphy, J. B.	Rockefeller Institute
Murphy, William P.	Boston, Mass.
Murray, Henry A.	Harvard University
Murray, Thomas J.	Rutgers College
Musser, John H.	Tulane University
Myers, Chester N.	Columbia University

Myers, Harold B.	University of Oregon
Myers, Victor C.	Western Reserve University

Necheles, H.	University of Chicago
Nelson, Erwin E.	University of Michigan
Nelson, Martha V.	State University of Iowa
Nelson, Thurlow C.	Rutgers College
Nelson, Victor E.	Iowa State College
Neuwirth, Isaac	New York University
Newburgh, Louis H.	University of Michigan
Ni, Tsang-gi	Peking Union Medical College, China
Nice, L. B.	Ohio State University
Nicholas, J. S.	Yale University
Nichols, M. S.	University of Wisconsin
Nielsen, Carl	Abbott Laboratories, Chicago
Niles, Walter L.	Cornell University Medical College
Noble, W. C., Jr.	Metropolitan Life Insurance Co.
Norris, Charles	Chief Medical Examiner, N. Y. City
Northrup, John H.	Rockefeller Institute, N. Y. City
Norton, J. F.	Herman Kiefer Hospital, Detroit
Novy, Frederick G.	University of Michigan
Nye, R. N.	Boston City Hospital

Obreshkove, Vasil	St. Stephen's College
Ochsner, E. W. A.	Tulane University
Oertel, Horst	McGill University
Olitsky, Peter K.	Rockefeller Institute, N. Y. City
Oliver, Jean	Lane Hospital, San Francisco, Calif.
Olmstead, W. H.	Washington University
Olmsted, J. M. D.	University of California
Ophüls, William	Stanford University Medical College
Opie, Eugene L.	Henry Phipps Institute, Philadelphia
Oppenheimer, B. S.	Columbia University
Ornstein, George G.	Columbia University
Osgood, E. E.	University of Oregon Medical School
Oslund, R. M.	University of Illinois Medical College
Osterhout, W. J. V.	Rockefeller Institute
Ottenberg, R.	Mt. Sinai Hospital, N. Y. City
Otto, H. L.	New York University and Bellevue Medical College

Packard, Charles	Columbia University
Page, Irvine H.	Munich, Germany
Palmer, Leroy S.	University of Minnesota
Palmer, W. L.	University of Chicago
Palmer, W. W.	Presbyterian Hospital, N. Y. City
Papanicolaou, George N.	Cornell University Medical College
Pappenheimer, A. M.	Columbia University
Park, E. A.	Johns Hopkins University
Park, William H.	N. Y. University and Bellevue Medical College
Parker, George H.	Harvard University
Parker, Julia T.	Columbia University
Pearce, Louise	Rockefeller Institute
Pearl, Raymond	Johns Hopkins University
Pease, Marshall C.	N. Y. Post-Graduate Medical School
Peirce, George J.	Stanford University Medical School
Pellini, Emil J.	N. Y. University and Bellevue Medical College
Pemberton, Ralph	Presbyterian Hospital, Philadelphia
Penfield, Wilder G.	Columbia University
Pepper, O. H. Perry	University of Pennsylvania
Perlzweig, William A.	Johns Hopkins Hospital
Permar, Howard H.	Mercy Hospital, Pittsburgh
Peskind, Samuel	Cleveland, Ohio

Peters, John P.	Yale University
Petersen, W. F.	University of Illinois Medical College
Petroff, S. A.	Trudeau Foundation, Saranac Lake, N. Y.
Pettibone, C. J. V.	University of Minnesota
Pfeiffer, J. A. F.	Johns Hopkins University
Pinner, Max	Maybury Sanitarium, Northville, Mich.
Plant, O. H.	University of Iowa
Plass, E. D.	University of Iowa
Plotz, Harry	Pasteur Institute, Paris
Pohlman, Augustus G.	St. Louis University
Pollock, L. F.	Northwestern University
Ponder, Eric	New York University
Powers, Grover F.	Yale University
Pratt, Fred H.	Boston University
Pratt, Joseph H.	Harvard University
Prewitt, Proviso	N. Y. University and Bellevue Medical College
Pribram, Ernest	St. Elizabeth's Hospital, Chicago
Prince, A. L.	Hartford Hospital, Conn.
Proescher, F.	Agnew State Hospital, Calif.
Pucher, G. W.	Hamden, Conn.

Quick, Armand J.	Philadelphia General Hospital
Quinby, W. C.	Peter Bent Brigham Hospital
Quinn, Edmond John	Columbia University

Ragins, Ida Kraus	University of Chicago
Raiziss, George W.	Research Institute of Cutaneous Medicine, Philadelphia
Rakestraw, Norris W.	Brown University
Ranson, S. W.	Northwestern University
Rapport, David	Western Reserve University
Rasmussen, A. T.	University of Minnesota
Ratner, Bret	N. Y. University and Bellevue Medical College
Ravenel, M. P.	University of Missouri
Ray, G. B.	Western Reserve University
Ray, Henry M.	South Side Hospital, Pittsburgh
Read, Bernard E.	Peking Union Medical College, China
Read, J. Marion	Stanford University Medical School
Reed, Carlos I.	University of Illinois Medical School
Reichert, F. L.	Stanford University
Reimann, Hobart A.	Peking Union Medical College, China
Reiman, Stanley P.	University of Pennsylvania
Rettger, L. F.	Yale University
Reynolds, Chapman	Tulane University
Reznikoff, Paul	Cornell University
Rhodenburg, George L.	Lenox Hill Hospital
Richards, Alfred N.	University of Pennsylvania
Richardson, Henry B.	Cornell University Medical College
Richey, DeWayne G.	University of Pittsburgh
Richter, Maurice	Columbia University
Riddle, Oscar	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Ringer, A. I.	Montefiore Hospital, N. Y. City
Ringer, Michael	Montefiore Hospital, N. Y. City
Robertson, H. E.	University of Minnesota
Robertson, Oswald H.	University of Chicago
Robertson, T. B.	University of Adelaide, South Australia
Robinson, Charles S.	Michigan Agricultural Station, Lansing
Robinson, Elliott	Antitoxin Laboratory, Boston
Robinson, G. Canby	Cornell University Medical College
Rockwood, Elbert W.	University of Iowa
Rogers, Fred T.	Baylor University
Rogoff, J. M.	Western Reserve University
Roman, Benjamin	Buffalo General Hospital, N. Y.
Rose, Anton R.	Prudential Life Insurance Co., Newark, N. J.

Rose, Mary Swartz	Columbia University
Rose, William C.	University of Illinois
Rosenau, M. J.	Harvard Medical School
Rosenow, E. C.	Mayo Foundation
Ross, Victor	Department of Health, N. Y. City
Roth, George B.	George Washington University
Rothschild, M. A.	Mt. Sinai Hospital, N. Y. City
Rous, Peyton	Rockefeller Institute
Rowe, Allan W.	Boston University

Sabin, Florence R.	Rockefeller Institute
Salant, William	University of Georgia
Salvesen, Harold A.	Rikshospitalet, Oslo, Norway
Sandburg, M.	Montefiore Hospital
Sanford, A. H.	Mayo Clinic
Sansum, W. D.	Santa Barbara Cottage Hospital
Scammon, R. E.	University of Minnesota
Schick, Bela	Mount Sinai Hospital, N. Y. City
Schlesinger, M. J.	Harvard University
Schloss, Oscar M.	Cornell University Medical College
Schlutz, F. W.	University of Minnesota
Schmidt, Carl F.	University of Pennsylvania
Schmidt, Carl L. A.	University of California
Schneider, Edward C.	Wesleyan University
Schultz, E. W.	Stanford University
Schultz, O. T.	University of Chicago
Schultz, W. H.	University of Maryland
Schwartz, Erich W.	Mellon Institute, Pittsburgh
Schwyzer, Fritz	Kastanienbaum, Switzerland
Scott, E. L.	Columbia University
Scott, F. H.	University of Minnesota
Scott, G. G.	College of the City of New York
Scott, R. W.	City Hospital, Cleveland, Ohio
Scott, W. J. M.	University of Rochester
Sears, H. J.	University of Oregon
Senior, Harold D.	N. Y. University and Bellevue Medical College
Shaffer, Philip A.	Washington University
Shaklee, A. O.	St. Louis University
Shannon, W. R.	University of Minnesota
Sharlit, Herman	Roosevelt Hospital, N. Y. City
Sharp, P. E.	Cornell University
Sheard, Charles	Mayo Clinic
Sherman, H. C.	Columbia University
Sherman, Hartley E.	Bureau of Science, Manila, P. I.
Sherman, James M.	Cornell University
Sherwin, Carl P.	Fordham University
Shevky, Eshref	Stanford University Medical College
Shibley, Gerald S.	Columbia University
Shiple, George J.	St. John's College, Toledo, Ohio
Shohl, Alfred T.	Western Reserve University
Shull, A. Franklin	University of Michigan
Shwartzman, Gregory	Mt. Sinai Hospital
Sia, Richard H. P.	Peking Union Medical College, China
Siler, J. F.	Balboa Heights, Canal Zone
Simonds, J. P.	Northwestern University
Sittenfield, M. J.	Columbia University
Smetana, Hans	Rockefeller Foundation
Smith, Arthur H.	Yale University
Smith, Carl H.	Cornell Medical College
Smith, Clarence	Bergenfield, New Jersey
Smith, Erma A.	Iowa State College
Smith, Frank F.	Harvard University
Smith, Fred M.	University of Iowa

Smith, George H.	Yale University
Smith, Millard	Simpson Memorial Institute, Ann Arbor, Mich.
Smith, Philip E.	Columbia University
Smith, Theobald	Rockefeller Institute, Princeton, N. J.
Smyly, H. Joselyn	Shantung Christian University, China
Snell, A. M.	Mayo Clinic
Snyder, Franklin F.	Johns Hopkins University
Sollmann, Torald	Western Reserve University
Somogyi, Michael	Jewish Hospital, St. Louis, Mo.
Soule, M. H.	University of Michigan
Sperry, Warren	University of Rochester
Sponsler, O. L.	University of California, Los Angeles
Stakman, E. C.	University of Minnesota
Stark, Mary B.	N. Y. Homeopathic Medical College, N. Y. City
Starkey, R. L.	Agricultural Exp. Station, New Brunswick, N. J.
Stenstrom, Wilhelm	University of Minnesota
Stevens, Franklin A.	Presbyterian Hospital, N. Y. City
Stewart, G. N.	Western Reserve Medical School
Stiles, Percy G.	Harvard University
Stillman, Edgar G.	Presbyterian Hospital, N. Y. City
Stillman, Ralph G.	Cornell University
Stockard, Charles R.	Cornell University Medical College
Stone, Calvin P.	Institute for Juvenile Research, Chicago
Stone, Leon S.	Yale University
Stookey, Lyman B.	University of Southern California
Storey, Thomas A.	Stanford University
Strong, Richard P.	Harvard University
Strouse, Solomon	Northwestern University
Sturgis, C. C.	Simpson Memorial Institute, Ann Arbor, Mich.
Suguira, Kanematsu	Memorial Hospital, N. Y. City
Sullivan, M. X.	Hygienic Laboratory, Washington, D. C.
Sullivan, Walter E.	University of Wisconsin
Sumner, James B.	Cornell University
Sundstroem, Edward S.	University of California
Sure, Barnett	University of Arkansas
Swain, R. E.	Stanford University
Swanson, W. W.	University of Minnesota
Sweany, Henry C.	University of Chicago
Sweet, J. Edwin	Cornell University Medical College
Swett, Francis H.	Vanderbilt University
Swift, H. F.	Rockefeller Institute, New York City
Swingle, W. W.	University of Iowa
Symmers, Douglas	N. Y. University and Bellevue Medical College

Tainter, Maurice L.	Stanford University
Talbot, Fritz B.	Harvard University
Tanner, Fred W.	University of Illinois
Tashiro, Shiro	University of Cincinnati
Tatum, A. L.	University of Wisconsin
Taylor, Charles V.	Stanford University
Ten-Broeck, Carl	Rockefeller Foundation, Princeton, N. J.
Terry, B. T.	Rochester, Minn.
Thalhimer, William	Michael Reese Hospital, Chicago
Thatcher, Robert W.	Massachusetts Agricultural College
Thienes, Clinton H.	University of Oregon
Thomas, Arthur W.	Columbia University
Thomas, J. E.	Jefferson Medical College
Thomas, Karl	Physiologisch-Chemisches Institut, Leipzig, Germany
Thomas, Walter S.	Clifton Springs Sanitarium, N. Y.
Thoraldsen, C. E.	New York Homeopathic Medical School
Thro, William C.	Cornell University Medical College
Tisdall, Frederick F.	University of Toronto
Tolstoi, Edward	New York City

Tomcsik, Joseph	Budapest, Hungary
Torrey, Harry B.	Stanford University Medical School
Torrey, J. C.	Cornell University Medical College
Trattner, H. R.	Western Reserve University
Traum, Jacob	University of California
Travis, L. E.	State University of Iowa
Tsen, Edgar T. H.	Epidemic Prevention Bureau, Peking, China
Tso, Ernest	Peking Union Medical College
Tunnicliff, Ruth	John McCormick Institute for Infectious Diseases, Chicago
Tuttle, W. W.	State University of Iowa
Tyzzer, E. E.	Harvard University

Uhlenhuth, Edward	University of Maryland
Underhill, Frank P.	Yale University

Van Allen, C. M.	University of Chicago
Van Dyke, H. B.	University of Chicago
Van Slyke, Donald D.	Rockefeller Institute
Vaughn, T. Wayland	Scripps Institution, California
Visscher, Maurice B.	University of Tennessee
Vogel, Karl M.	St. Luke's Hospital, New York City
Von Meysenburg, Ludo	Tulane University
Von Oettingen, W. F.	Western Reserve University

Wadsworth, Augustus B.	N. Y. State Department of Health
Wakerlin, G. E.	University of Chicago
Waksman, S. A.	N. J. State Agr. Experiment Station
Wallace, George B.	N. Y. University and Bellevue Medical College
Wallin, Ivan E.	University of Colorado
Walton, D. C.	Edgewood Arsenal, Edgewood, Md.
Wang, Chi Che	University of Chicago
Wangenstien, Owen H.	University of Minnesota
Warthin, Alfred S.	University of Michigan
Wasteneys, H.	University of Toronto
Watanabe, C. K.	Watanabe Hospital, Tokyo, Japan
Waud, R. A.	University of Western Ontario, Canada
Wearn, J. T.	Boston City Hospital
Webster, J. P.	Presbyterian Hospital, New York City
Webster, Leslie T.	Rockefeller Institute
Weiskotten, Herman G.	Syracuse University
Weiss, Charles	Washington University
Weiss, Harry	Columbia University
Weiss, Soma	Boston City Hospital
Welker, W. H.	University of Illinois Medical College
Weller, Carl V.	University of Michigan
Wenner, William F.	State University of Iowa
Werkman, Chester H.	Iowa State College
West, Edward S.	Washington University
West, Randolph	Presbyterian Hospital, N. Y. City
Weymouth, Frank W.	Stanford University
Wheeler, William M.	Bussey Institute
Whipple, George H.	University of Rochester
White, G. Benjamin	Antitoxin and Vaccine Laboratory, Boston
White, H. L.	Washington University
Wible, Charles L.	University of Nebraska
Wiggers, Carl J.	Western Reserve University
Wilhelmj, C. M.	Mayo Foundation
Willaman, J. J.	Agricultural Exp. Station, Geneva, N. Y.
Williams, Anna W.	Department of Health, N. Y. City
Williams, Horatio B.	Columbia University
Williams, H. U.	University of Buffalo
Williams, J. R.	Highland Hospital, Rochester, N. Y.
Williams, Robert R.	Western Electric Company

Williams, Roger J.	University of Oregon
Williamson, Charles S.	University of Illinois
Willier, Benjamin H.	University of Chicago
Wilson, D. Wright	University of Pennsylvania
Wilson, Edmund B.	Columbia University
Wilson, Frank N.	University of Michigan
Wilson, James R.	Cornell University Medical College
Winslow, C. E. A.	Yale University
Winternitz, Milton C.	Yale University
Witschi, Emil	State University of Iowa
Wolbach, S. B.	Harvard University
Wolf, C. G. L.	Addenbrooke's Hospital, Cambridge, England
Wollstein, Martha	Babies' Hospital, N. Y. City
Wood, Francis C.	Columbia University
Woodruff, L. L.	Yale University
Wu, Hsien	Peking Union Medical College, China
Wulzen, Rosalind	University of Oregon

Yatsu, Naohide	Zoological Institute, Tokyo, Japan
Yerkes, Robert M.	Yale University
Youland, William E., Jr.	N. Y. Homeopathic Medical College
Young, C. C.	Department of Health, Mich.
Yudkin, Arthur M.	Yale University

Zinsser, Hans	Harvard University
Zucker, Theodore F.	Columbia University

MEMBERS' LIST (By Branches)

Honorary Members.

Councilman, W. T., Harvard University.
Halliburton, W. D., London, England.
Lombard, Warren P., University of Michigan.
Porter, William, Harvard University.
Reichert, E. T., University of Pennsylvania.
Richet, Charles, Paris, France.
Schaffer, E. S., Edinburgh, Scotland.
Von Müller, F., Munich, Germany.
Welch, W. H., Johns Hopkins University.

New York Society

Greater New York.

Carnegie Institute—Banta, A. M., Davenport, C. B., Laughlin, H. H., MacDowell, E. C., Riddle, O.

College of the City of New York—Goldforb, A. J., Harrow, B., Scott, G. G.

College of Physicians and Surgeons, Columbia University—Berg, B. N., Clarke, H. T., Copenhaver, W. M., Detwiler, S. R., Draper, G., Engle, E. T., Flinn, F. B., Foster, G. L., Gay, F. P., Gies, W. J., Hopkins, J. G., Jobling, J. W., Krasnow, F., Lee, F. S., Lieb, C. C., Miller, E. G., Jr., Mulinos, M. G., Myers, C. N., Oppenheimer, B. S., Ornstein, G. G., Pappenheimer, A. M., Parker, J. T., Penfield, W. G., Richter, M., Scott, E. L., Sittenfield, M. J., Smith, P. E., Thomas, A. W., Weiss, H., Williams, H. B., Zucker, T. F.

Columbia University—Calkins, G. N., Coulter, C. B., Dunn, L. C., Eddy, W. H., Gregory, L. H., Heft, H. L., Lancefield, D. E., MacLeod, G., Packard, C., Quinn, E. J., Sherman, H. C., Rose, M. S., Wilson, E. B., Wood, F. C.

Cornell University Medical College—Bagg, H. J., Behre, J. A., Benedict, S. R., Blau, N., Cattell, M., Cecil, R. L., Chambers, W. H., Churchman, J. W., Coca, A. F., DuBois, E. F., Edwards, D. J., Elser, W. J., Ewing, J., Foster, N., Gold, H., Hartwell, J. A., Hatcher, R. A., Kahn, M. C., Levine, S. Z., Loebel, R. O., Lusk, G., Miles, W. L., Papanicolaou, G. N., Reznikoff, P., Richardson, H. B., Robinson, G. C., Schloss, O. M., Smith, C. H., Stillman, R. G., Stockard, C. R., Sweet, J. E., Thro, W. C., Torrey, J. C., Wilson, J. R.

Montefiore Hospital—Baumann, E. J., Brand, E., Levine, M., Marine, D., Ringer, E. I., Ringer, E. M., Sandberg, M.

Mt. Sinai Hospital—Baehr, G., Crohn, B. B., Elsberg, C. A., Epstein, A. A., Frank, R. T., Gross, L., Kohn, J. L., Ottenberg, R., Rothschild, M. A., Schick, B., Shwartzman, G.

New York Homeopathic Medical College—Danzer, C. S., Kleiner, I. S., Stark, M. B., Thoraldsen, C. E., Youland, W. E.

New York University and Bellevue Medical College—Barber, W. H., Brooks, H., Chambers, R., Coombs, H. C., Coleman, W., DeGraff, A. C., Eisberg, H. B., Fry, H. J., Gettler, A. O., Hess, A. F., Howland, R. B., Krumweide, C., Mandel, A. R., Neuwirth, I., Noble, W. C., Jr., Otto, H. L., Park, W. H., Pellini, E. J., Ponder, E., Prewitt, R. V., Ratner, B., Semon, H. D., Symmers, D., Wallace, G. B.

New York Post-Graduate Medical School—Bailey, C. V., Cohn, M., Chace, A. F., Crampton, C. W., Halsey, R. H., Kast, L., Katayama, I., Killian, J. A., MacNeal, W. J., Mosenthal, H. P., Marshall, C., Jr.

Presbyterian Hospital—Atchley, D. W., Bauman, L., Dochez, A. R., Geyelin, H. R., Heidelberger, M., Ladd, W. S., Loeb, R. F., Levy, R. L., Meleney, F. L., Palmer, W. W., Stevens, F. A., Stillman, E., Shibley, G. S., Webster, J. P., West, R.

Princeton University—Conklin, E. G., Harvey, E. H., Jones, F. S., Smith, T.

Rockefeller Institute—Anson, M. L., Avery, O. T., Bauer, J. H., Binger, C. A. L., Blinks, L. R., Brown, W. H., Cohn, A. E., Cole, R., Doan, C. A., Flexner, S., Florence, L., Forkner, C. E., Frobischer, M. J., Gates, L., Hannon, R. R., Hill, S. E., Irwin, M., Jacobs, W. A., Landsteiner, K., Levene, P. A., Lewis, P. A., Liu, S., Long, P. H., Lynch, C. J., MacKay, E. M., MacMaster, P. D., Mirsky, A. E., Muckenfuss, R. S., Murphy, J. B., Murray, H. A., Jr., Northrop, J. H., Olitsky, P. K., Osterhout, W. J. V., Pearce, L., Rous, P., Sabin, F. R., Swift, H. F., Ten Broeck, C., VanSlyke, D. D., Webster, L. T.

Rutgers University—Anderson, J. F., Cole, W. H., Murray, T. J., Nelson, T. C., Waksman, S. A.

Yale University—Anderson, R. J., Baitsell, G. A., Blake, E. G., Burr, H. S., Chittenden, R. H., Cowgill, G. R., Gross, E. G., Harrison, R. G., Harvey, S. C., Himwich, H. E., Hussey, R., Johnson, T. B., Mendel, L. P., Moise, T. S., Morse, A., Nicholas, J. S., Peters, J. P., Powers, G. F., Rettger, L. F., Smith, A. H., Smith, G. H., Stone, L. S., Underhill, F. P., Woodruff, L. L., Winslow, C. E. A., Winternitz, M. C., Yerkes, R. M., Yudkin, A. M.

Miscellaneous—Baldwin, W. M., Banzhaf, E. J., Benton, A. G., Blakeslee, A. F., Blatherwick, N. R., Berg, W. N., Bernhard, A., Bodansky, A., Boots, R. H., Burton-Opitz, R., Byrne, J., Clark, G. W., Collens, W. S., Curtis, M. R., Dakin, H. D., Draper, J. W., Dresbach, M., Dubin, H. E., Eggston, A. A., Falk, K. G., Famulener, L. W., Field, C. W., Fishberg, E. H., Frankel, F. H., Freedman, L., Gager, C. S., Garbat, A. L., Gardner, L. U., Goldberg, S. A., Goldbloom, A. A., Greenwald, I., Harris, I. F., Harris, R. G., Hawk, P. B., Hooper, C. W., Jacobsen, W. C., Jaffe, H. L., Kahn, M. H., Kirkbridge, M. B., Knudson, A., Koch, M. O., Kopeloff, N., Korns, J. H., Kramer, B., Kugelmass, I. N., Lambert, R. A., Lamson, R. W., Leonard, C. S., Levin, I., Lyle, W. G., Mackenzie, G. M., Maltaner, F., Mann, H., Master, A. M., Mavor, J. W., Medlar, E. M., Meigs, E. B., Norris, C., Obreskove, U., Petroff, S. A., Prince, A. L., Pucher, G. W., Rohdenburg, G. L., Rose, A. R., Ross, V., Roth, G. B., Schneider, E. C., Schultz, W. H., Sharlit, H., Sherwin, C. P., Smetana, H., Smith, C. A., Saguira, K., Starkey, R. L., Tolstoi, E., Vogel, K. M., Willaman, J. J., Williams, A. W., Williams, R. R., Wollstein, M.

Outside Greater New York.

Alabama—Brooks, C., Larson, E.

Arkansas—Sure, B.

Colorado—Corper, H. J., Hall, I., Wallin, I. E.

District of Columbia—Branham, S. E., Csonka, F. A., DeEds, F., Holm, G. E., Howe, P. E., Leake, J. P., Siler, J. F., Sullivan, M. X.

Georgia—Lamar, B. V., Salant, W.

Indiana—Bills, C. E., Clowes, G. H. A.

Kentucky—Barbour, H. G., Beutner, R.

Maryland—Johns Hopkins University—Abel, J. J., Amoss, H. L., Brown, J. H., Bull, G., Carter, E. P., Chen, K. K., Cohen, B., Dunn, H. L., Geiling, E. M. K., Harrop, G. A., Hill, E. C., Holt, L. E., Jr., Howell, W. H., Jennings, H. S., Larson, J. A., Lee, F. C., Longcope, W. T., McCollum, E. V., Macht, D. I., Marshall, E. K., Jr., Meyer, A., Meyer, A. L., Michaelis, L., Park, E. A., Pearl, R., Perlzweig, W. A., Pfeiffer, J. A., Snyder, F. F., Welch, W. H.

Miscellaneous—Deuel, H. J., Jr., Hamilton, B. K., Hemmeter, J. C., Uhlenhuth, E.

Massachusetts—Boston City Hospital—Mallory, F. B., Muller, G. L., Nye, R. N., Minot, G. R., Weiss, S.

Harvard University—Aub, J. C., Blackfan, K. D., Blum, H. F., Cannon, W. B., Cohn, E. J., Christian, H. A., Crozier, W. J., Dawson, J. A., Ferry, R. M., Felton, L. D., Fitz, R., Forbes, A., Gamble, J. L., Hale, W., Hoskins, R. G., Hunt, R., Jackson, H., Jr., Lee, M. O., McIver, M. A., Mendenhall, W. L., Mueller, J. H., Murray, H. A., Parker, G. H., Pratt, J. H., Quinby, W. C., Rosenau, M. J., Schlesinger, M. J., Smith, F. F., Stiles, P. G., Strong, R. P., Talbot, F. B., Tyzzer, E. E., Wearn, J. T., Wolbach, S. B., Zinsser, H.

Miscellaneous—Blumgart, H. L., Derick, C. L., Forbes, H., Glaser, O. C., Hooker, S. B., Joslin, E. P., McCrudden, F. H., Millet, J. A., Murphy, W. P., Pfaff, F., Pratt, F. H., Robinson, E. S., Rowe, A. W., Thatcher, R. W., Wheeler, W. M., White, B.

Michigan—University of Michigan—Bunting, R. W., Christman, A. A., Eckstein, H. C., Edmunds, C. W., Gesell, R., Hadley, P. B., Heilbrunn, L. V., Huber, G. C., Lewis, H. B., Little, C. C., Lombard, W. P., McGinty, D. A., Nelson, E. E., Newburgh, L. H., Novy, F. G., Roseboom, B. B., Shull, A. F., Soule, M. H., Warthin, A. S., Weller, C. V., Wilson, F. N.

Miscellaneous—Fine, W. S., Gaebler, O. H., Isaacs, R., Kahn, R. L., McQuarrie, I., Norton, J. F., Pinner, M., Robinson, C. S., Smith, M., Sturgis, C. C., Young, C. C.

Nebraska—Guenther, A. E., Levine, V. E., Wible, C. L.

North Carolina—Davison, W. C., Dienes, L., MacNider, W. deB.

Ohio—University of Cincinnati—Fischer, M. H., Jackson, D. E., Shiro, T.

Western Reserve University—Barlow, O., Beard, H. H., Collett, M. E., Crile, G. W., Cutler, E. C., Ecker, E. E., Goldblatt, H., Karsner, H. T., Katz, L. N., Kline, B. S., Megrail, E., Myers, V. C., Rapport, D., Ray, G. B., Rogoff, J., Scott, R. W., Shohl, A. T., Sollmann, T., Stewart, G. N., Trattner, H. R., Von Oettingen, W. F., Wiggers, C. J.

Miscellaneous—Brown, J. B., Hitchcock, F. A., Lyman, J. F., Nice, L. B., Peskind, S., Shiple, G. J.

Oregon—University of Oregon—Allen, W. F., Burget, G. E., Huestis, R. R., Larsell, O., Manville, F. A., Menne, F. R., Moore, A. R., Moore, M. M., Myers, H. B., Osgood, E. E., Sears, H. J., Thienes, C. H., Williams, R. J., Wulzen, R.

Pennsylvania—University of Pennsylvania—Abbott, A. C., Austin, J. H., Bazett, H. C., Bergey, D. H., Bodine, J. H., Bronk, D. W., Drabkin, D. L., Goldschmidt, S., Jacobs, M. H., Jonas, L., Kolmer, J. A., Krumbhaar, E. B., Lucke, B., McCutcheon, M., Mudd, S., Pepper, O. H. P., Raiziss, G. W., Reichert, E. T., Reimann, S. P., Richards, A. N., Schmidt, C. F., Wilson, D. W.

University of Pittsburgh—Collins, H. H., Donaldson, J. C., Guthrie, C. G., Haythorn, S., Hooker, D., Kruse, T. K., McEllroy, W. S., McMeans, J. M., Menten, M. L., Permar, H. H., Ray, H. M., Richey, D. G.

Miscellaneous—Coghill, G. E., Donaldson, H. H., Dutcher, R. A., Freund, J., Furth, J., Githens, T. S., Hoffmann, G. L., Huntoon, F. M., King, H. D., Looney, J. M., Mellon, R. R., Morse, W., Opie, E. L., Pemberton, R., Quick, A. J., Schwartz, E. W., Thomas, J. E.

Rhode Island—Rakestraw, N. W.

South Carolina—Collins, K. R.

South Dakota—Atkinson, H. V.

Tennessee—Cullen, G. E., Cunningham, R. S., Garrey, W. E., Lamson, P. D., Mason, K. E., Meleney, H. E., Swett, T. W., Terry, B. T., Visscher, M. B.

Texas—Hartman, C., Hendrix, B. M., Lund, E. J., Muller, H. J., Reed, C. I., Rogers, F. T.

Virginia—Horsley, J. S., Jordan, H. E.

Washington—Guberlet, J. E.

West Virginia—Chidester, F. E.

Branches

Illinois Branch.

University of Chicago—Bloom, W., Cannon, P. R., Carlson, A. J., Child, C. M., Curtis, G. M., Dack, G. M., Falk, I. S., Gerard, R. W., Greer, J. R., Hamburger, W. W., Hanke, M. E., Hastings, A. B., Hiller, F., Hinrichs, M. A., Johnson, C. A., Jordan, E. O., Kleitman, N., Koch, F. C., Kunde, M. M., Leiter, L., Lillie, F. R., Lillie, R. S., Luckhardt, A. B., McLean, F., Miller, C. P., Jr., Necheles, H., Palmer, W. L., Ragins, I. K., Robertson, O. H., Sweany, H. C., Tatum, A. L., Van Allen, C. M., Van Dyke, H. B., Wakerlin, G. E., Willier, B. H.

University of Illinois—Andrews, E., Bachem, A., Bergeim, O., Boyden, E. A., Davis, D. J., Falls, F. H., Jaffe, R. H., Keeton, R. W., Koch, E. M., Koser, S. A., Levinson, S. A., Oslund, R. M., Petersen, W. F., Reed, C. I., Rose, W. C., Tanner, F. W., Welker, W. H., Williamson, C. S.

Northwestern University—Day, A. A., Dragstedt, C. A., Dragstedt, L. R., Farmer, C., Ivy, A. C., Kendall, A. I., Pollock, L. F., Ranson, S. W., Simonds, J. P., Strouse, S.

Miscellaneous—Adler, H. M., Albus, W. R., Austin, W. C., Arnold, L., Boyd, T. E., Buchbinder, W. C., Fisher, N. F., Givens, M. H., Howell, K. M., Lashley, K. S., Locke, A. P., McJunkin, F. A., Miller, J. L., Nielsen, C., Pribram, E., Schultz, O. T., Stone, C. P., Thalhimer, W., Tunnicliff, R., Wang, C. C.

Iowa Branch.

State University of Iowa—Boyd, J. D., Brown, L. A., Daniels, A. L., Gibson, R. B., Hansmann, G. H., Helff, O. M., Hines, H. M., Jeans, P. C., Loehwing, W. F., McClintock, J. T., Mattill, H. A., Miller, G. H., Nelson, M. V., Plant, O. H., Plass, E. D., Rockwood, E. W., Smith, F. M., Swingle, W. W., Travis, L. E., Tuttle, W. W., Wenner, W. F., Witschi, E.

Iowa State College—Becker, E. R., Buchanan, R. E., Fulmer, E. I., Hammer, B. W., Levine, M., Melhus, I. E., Nelson, V. E., Smith, E. A., Werkman, C. H.

Minnesota Branch.

University of Minnesota—Amberg, S., Bell, E. T., Berglund, H., Bollman, J. L., Boothby, W. W., Brown, E. D., Eckles, C. H., Fahr, G., Fitch, C. P., Gortner, R. A., Green, R. G., Greisheimer, E., Harris, J. A., Henrici, A. T., Hirschfelder, A. D., Jackson, C. M., Kendall, E. C., Larson, W. P., Lyon, E. P., McClendon, J. F., Medes, G., Palmer, L. S., Pettibone, C. J. V., Rasmussen, A. T., Rosenow, E. C., Sanford, A. H., Scammon, R. E., Schlutz, F. W., Scott, F. H., Shannon, W. R., Stakman, E. C., Stenstrom, W., Swanson, W. W., Wangensteen, O. H.

Miscellaneous—Alvarez, W. C., Greene, C. H., Helmholz, H. F., Hench, P. S., Higgins, G. M., Magath, T. B., Mann, F. C., Robertson, H. E., Sheard, C., Snell, A. M., Terry, B. T., Wilhelmj, C. M.

Missouri Branch.

St. Louis University—Auer, J., Briggs, A. P., Bulger, H. A., Collier, W. D., Doisy, E. A., Fleisher, M. S., Graves, W. W., Griffith, W. H., Hertzman, A. B., Key, J. A., Kinsella, R. A., Kuntz, A., Pohlman, A. G., Shaklee, A. O.

University of Missouri—Allen, E., Ravenel, M. P.

Washington University—Alexander, H. L., Barr, D. P., Bishop, G. H., Bronfenbrenner, J., Cooke, J. V., Cowdry, E. V., Erlanger, J., Gasser, H. S., Graham, E. A., Gruber, C. M. M., Hanson, F. B., Howard, H. J., Loeb, L., McCordock, H. O., Marriott, M., Olmstead, W. H., Shaffer, P. A., Weiss, C., West, E. S., White, H. L.

Miscellaneous—Broun, G. O., Ellis, M. M., Jorstad, L. H., Somogyi, M.

Pacific Coast Branch.

University of California—Ball, G. H., Beckwith, T. D., Brooks, M. M., Brooks, S. C., Burnett, T. C., Cook, S. F., Daniel, J. F., Dunn, M. S., Eberson, F., Gahl, R., Greenberg, D. N., Evans, H. M., Holmes, S. J., Kellogg, W. H., Kessel, J. F., Kofoid, C. A., Leake, C. D., Lipman, C. B., Lucas, W. P., Marshall, J. A., Marshall, M. S., Meyer, K. F., Moore, L. M., Mudge, C. S., Olmsted, J. M. D., Schmidt, C. L. A., Sponsler, O. L., Sundstroem, E. E., Traum, J.

Stanford University—Addis, T., Alsberg, C. L., Barnett, G. D., Baumberger, J. P., Bloomfield, A. L., Cowan, J. F., Danforth, C. H., Dickson, E. C., Dock, W., Faber, H. K., Gurchot, C., Hanzlik, P. J., Holman, E., Jungeblut, C., Luck, J. M., Manwaring, W. H., Martin, E. G., Meyer, A. W., Mehrtens, H. G., Miles, W. R., Oliver, J., Ophüls, W., Pierce, G. J., Read, J. M., Reichert, F. L., Schultz, E. W., Shevky, E., Storey, T., Swain, R. E., Tainter, M. L., Taylor, C. V., Torrey, H. B., Weymouth, F. W.

Miscellaneous—Allen, B. M., Anderson, E. G., Becking, L. B., Burrows, M. T., Hurwitz, S. H., Langstroth, L., MacDougal, D. T., Morgan, T. H., Proescher, F., Sansum, W. D., Stookey, L. B., Vaughan, T. W.

Peking Branch.

Peking Union Medical College—Cash, J. R., Cheer, S. M., Chou, T., Dieuaide, F. R., Fortuyn, A. B. D., Keefer, C. S., Lim, R. K., Mills, C. A., Ni, T., Read, B. E., Reimann, H. A., Sia, R. H. P., Tsen, E. T. H., Tso, E., Wu, H.

Shantung Christian University—Smyly, H. J.

Southern Branch.

Tulane University—Ashman, R., Bass, C. C., Castellani, A., Cohn, I., Corley, R. C., Couret, M., Cummins, H., Duval, C. W., Faust, E. C., Gould, H. N., Hafkesbring, H. R., Halsey, J. T., Hardesty, I., Harris, W. H., Hathaway, E. S., Herrmann, G. R., Johns, F. M., Laurens, H., Mayerson, H. S., Meysenburg, L. von, Matas, R., Musser, J. H., Jr., Ochsner, E. W. A., Reynolds, C.

Western New York Branch.

University of Buffalo—Atwell, W. J., Bowen, B. D., Griffith, F. R., Hartman, F. A., Lathrop, C. O., Roman, B., Williams, H. U.

Cornell University—Carpenter, C. M., Fish, P. A., Hagan, W. A., Hayden, C. E., Liddell, H. S., Maynard, L. A., Sharp, P. F., Sherman, J. M., Sumner, J. B.

University of Rochester—Bayne-Jones, S., Birkhaug, K. E., Bloor, W. R., Burns, R. K., Jr., Claussen, S. W., Corner, G. W., Dye, J. A., McCann, W. S., McQuarrie, I., Murlin, J. R., Scott, W. J. M., Sperry, W. M., Whipple, G. H.

Syracuse University—Brewer, R. K., Dooley, M. S., Knowlton, F. P., Koppanyi, T., Mitchell, O. W. H., Weiskotten, H. G.

Miscellaneous—Cori, C. F., Hubbard, R. S., Thomas, W. S., Williams, J. R.

Wisconsin Branch.

University of Wisconsin—Bardeen, C. R., Bast, T. H., Bradley, H. C., Bunting, C. H., Clark, P. F., Cole, L. J., Daniels, F., Duggar, B. M., Eyster, J. A. E., Fred, E. B., Guyer, M. F., Hastings, E. J., Hisaw, F., Keitt, G. W., Lorenz, W. F., Meek, W. J., Muehlberger, C. W., Nichols, M. S., Sullivan, W. E., Tatum, A. L., Walton, D. C.

Foreign.

Austria—Bruecke, E.

Belgium—Funk, C., Heymans, C.

Canada—Caulfield, A. H. W., Collip, J. B., Fitzgerald, J. G., Holman, W. L., Hunter, A., Irving, L., Klotz, O., Macallum, A. B., Oertel, H., Tisdall, F. F., Wasteneys, H., Waud, R. A.

Denmark—Fridericia, L. S., Lundsgaard, C.

England—Davies, H. W., Fulton, J. F., Gaskell, J. F., Wolf, C. G. L.

France—May, R. M., Plotz, H.

Germany—Balls, A. K., Erdman, R., Fischer, A., Mueller, E. F., Page, I. H., Thomas, K.

Hungary—Tomcsik, J.

India—Cruickshank, E. W. H.

Japan—Shink, H., Watanabe, G. R., Yatsu, N.

Palestine—Kligler, I. J.

Philippine Islands—Sherman, H. E.

Porto Rico—McKinley, E. B.

Norway—Salvesen, H. A.

Scotland—MacLeod, J. J. R.

South Australia—Robertson, T. B.

Switzerland—Asher, L., Schwyzer, F.

Syria—Hinshaw, H. C.

AUTHORS' INDEX

(*The numerals indicate the page.*)

Abramson, H. A. Orientation of cylindroid particles of gelatin-serum gels, 147; Ionization of adsorbed protein, 689.
Adams, M. A. See Himwich, H. F., 120.
Adams, W. E., VanAllen, C. M., and Day, L. A. Intratracheal inoculation on chronicity of lung abscess, 163.
Albi, R. W., and Boyd, T. E. Histamine absorption from intestine in parathyroidectomy, 768.
Alkourie, L. E. See Larson, E., 210.
Allison, C. B., and Hubbard, R. S. Urobilinogen concentration in urine containing bilirubin, 629; see Hubbard, R. S., 438.
Allumbaugh, H. R. Blood of mother and new born, 814.
Alper, J. M. See Thompson, W. O., 697.
Anchel, D. See Bogert, M. T., 721; see Boyd, L. J., 570.
Anderson, O. D. See Liddell, H. S., 81.
Anderson, W. E. See McAmis, A. J., 515.
Andrews, E. See Reuterskiold, K., 17.
Ane, J. N. See Menville, L. J., 758.
Arnold, L., and Hull, T. G. Diarrhea and gastro-intestinal auto-disinfection, 159.
Avery, O. T. See Julianelle, L. A., 224, 226, 227.
Azevedo, J. L. See Manwaring, W. H., 525.

Bachem, A. Ultraviolet transparency of layers of skin, 778; see Jaffe, R. H., 166.
Bahrs, A. M. Growth-promoting power of digestive mucosa, 503.
Baldes, E. J., and Corbeille, C. Photographic method of recording plethysmograms, 711.
Baldridge, C. W. See Barer, A., 872.
Baldwin, W. M., and Nelson, W. C. Histologic effects of high frequency currents, 588.
Barbour, H. G. See Flowers, S. H., 572.
Barer, A., and Baldridge, C. W. Liver, and basal metabolism in anemia, 872.
Baruch, H. B. See Curphey, T. J., 687.
Bassett, R. B. See Loeb, L., 860.
Baumann, E. J. See Marine, D., 822.
Bayne, T. L., Winsor, A. L., and Winters, E. S. Conditioned motor responses, 342.
Bean, J. W. High oxygen pressures on blood acidity, flow and respiration, 832.
Beard, H. H., and Myers, V. C. Blood regeneration in nutritional anemia, 510; Pomerene, E., and Shipley, R. A. Effect of hydroquinone in Vitamin A deficiency, 328.
Beard, P. J. Resistance of purified bacteriophages to disinfectants, 880.
Beard, S. D., Clark, G. W., and Moses, M. J. Standardization of liver extract, 13.
Beck, M. D. Coccidicides immitis in lesions, 534.
Becks, H. Electrolytes of saliva, 93.
Beers, D. N. Return of vision in transplanted amphibian eyes, 477.
Berglund, H., and Chang, H. C. Achlorhydria during fever and histamine test, 422; Keefer, C. S., and Yang, C. S. Deficiency anemia and cod liver oil, 418; see Yang, C. S., 417.
Berkson, J. See Flexner, L. B., 592.
Bieter, R. N. Splanchnic nerves on glomerular blood flow and mechanism of reflex aneuria, 792.
Birch, C. L., and Boyden, E. A. Tonus changes in gall bladder by Faradic stimulation, 314; Gall bladder and Faradic stimulation of stomach, 466; see Boyden, E. A., 312.
Bishop, G. H., and Heinbecker, P. Correlation between threshold and conduction rate in myelinated nerves, 241; see Heinbecker, P., 645.
Biskind, M. S. See Dan, M., 52.
Blackberg, S. N. Radiation and resistance to tetanus toxin in rickets, 757; Vitamin A and B content of sweet potatoes, 254; see Menville, L. J., 758.
Blanchard, E. W. See Wenner, W. F., 182.
Blier, Z. A. Reciprocal innervation during pupillary dilatation, 461.
Blinks, L. R. Resistance changes in collodion membranes and cells, 358; Harris, E. S., and Osterhout, W. J. V. Stimulation in Nitella, 836.
Bloom, W. Development of fibers in cultures of embryonic heart muscle, 779.
Bloomfield, E. L. See Rose, M. S., 322.
Blount, R. F. Seasonal variations in interstitial tissue of testis, 727.
Blumenthal, R. See Slifer, E. H., 605.
Bogert, M. T., Anchel, D. and Husted, H. G. Pharmacological reactions of benzothiazole, 721.
Bollas, G. D. See Tanner, F. W., 154.
Boone, T. H. See Manwaring, W. H., 525.
Boyd, J. D. Endogenous rickets, 181; see Magee, C., 718; see Stearns, G., 717.
Boyd, L. J., Brody, J. G. and Anchel, D. Experimental cardiac arrhythmias, 570.
Boyd, T. E. See Albi, R. W., 768; see Mammoser, L. F., 765.
Boyden, E. A., and Birch, C. L. Emptying of gall bladder in children, 312; see Birch, C. L., 314, 466.
Brassfield, C. R. Peripheral chemical control of pulmonary ventilation, 833.
Brody, J. G. See Boyd, L. J., 570.
Bronfenbrenner, J. See Hetler, D. M., 644.
Brooks, M. M. Penetration of methylene blue and trimethyl thionine into living cells, 290.
Broun, G. O. Test for mechanical fragility of blood, 733; see Gerard, J., 209.
Brown, J. B., and Rawlins, A. L. Effect of feeding whale oil on depot fat, 704.
Buchanan, R. E. See Fulmer, E. I., 446.
Bump, W. S., and Crowe, S. M. Uretero-ureteral anastomosis, 18.
Bumstead, J. H. See Rich, A. R., 397.
Bunney, W. E., and White, B. Buffered diluent for Schick toxin, 801.
Burr, G. See McClendon, J. F., 265, 430, 782.
Burrows, M. T. Nutrition and development of cancer, 574.
Butler, E. G. Thyroid and rate of cell division, 231.

Campbell, J. R., Jr. See Smith, M., 395.

Cannon, P. R., and McClelland, P. H. Ectoparasites in Bartonella infection 157; see Stuppy, G. W., 314.

Cary, W. E. See Dack, G. M., 49.

Casey, A. E. Basophilia as index of resistance, 670; see Pearce, L., 666, 668.

Castellani, A. A mannitol fermenting Monilia, 544; Mannitol agar in differentiation of fungi of type Blastomyces, 544; A new strain of meta-dysentery bacilli, 545; A new vibriothrix, 543.

Cecil, R. L., Nicholls, E. E., and Stainsby, W. J. Bacteriology of blood and joints in chronic infectious arthritis, 6.

Chamberlain, B. Experimental hyperthyroidism and sympathetic irritability, 459.

Chambers, W. H. See Himwich, H. E., 120.

Chang, H. C. See Berglund, H., 422; See Lim, R. K. S., 271.

Chen, K. K., Wu, C. K., and Henriksen, E. Ephedrine homologs and isomers, pharmacological action and constitution, 348; and Jensen, H. Crystalline principles from Chan Su, dried venom, 378.

Chou, T. Alkaloids from Corydalis ambigua, 735.

Churchman, J. W. Gram structure of cocci, 364.

Chidester, F. E., and Eaton, A. G. Arsenic, sulphates and vitamin A-free diet with iron, 141; Eaton, A. G., and Thompson, C. P. Arsenic and vitamin A-free diet with iron iodide, 143.

Christensen, K. Effect of castration on oviduct, 652.

Cipra, A. See Marine, D., 822.

Clark, E. See Cunningham, B., 221.

Clark, G. W. See Beard, S. D., 13.

Claus, P. E. See Guyer, M. F., 65.

Clausen, E. M. L. Infra-red radiation and rickets, 77.

Clausen, H. J. See Helff, O. M., 650.

Clowes, G. H. A. See Zerfas, L. G., 399.

Cohen, P. See Karelitz, S., 11.

Collens, W. S., and Murlin, J. R. Hyperglycemia following portal injection of insulin, 485.

Conklin, C. See McClendon, J. F., 265.

Cook, S. F., and Rose, M. I. Changes in size of spleen after exercise, 529.

Corbeille, C. See Baldes, E. J., 711.

Cordonnier, J. KI and thyroid on metabolic rate, 636.

Cori, C. F., and Cori, G. T. Epinephrine and blood sugar utilization, 345; Fate of glucose in eviscerated animal, 432; and Goltz, H. L. Glucose absorption from intestinal tract, 433.

Cori, G. T. See Cori, C. F., 345, 432, 433.

Corley, R. C. Determination of levulose in blood and urine, 248; Metabolism, and galactose and glucose, 694; Tolerance for levulose in experimental liver injury, 249.

Crandall, L. A., and Walsh, E. L. Iodized oils and serous membranes, 307.

Crowe, S. M. See Bump, W. S., 18.

Csonka, F. A. See Phillips, M., 320.

Cunningham, B., Clark, E., and Lee, M. O. Effects of aconitine, 221.

Cunningham, R. S. See Lawrence, J. S., 331.

Curphey, T. J., and Baruch, H. B. Immunization by type specific pneumococcic pleural exudates, 687.

Curtis, G. M., and Huggins, C. H. Control of ascites, 70; and Pacheco, G. A. Experimental hypochloremia, 874.

Cutler, E. C. See Herrmann, L. G., 28.

Cutler, J. T. See Minot, A. S., 138, 607.

Dack, G. M., Cary, W. E., and Harmon, P. H. Experimental paratyphoid intoxication, 49; Jordan, E. O., and Wood, W. L. Experimental food-poisoning with living paratyphoid bacilli, 301.

Dan, M., and Biskind, M. S. Automatic drop recorder, 52.

Danforth, C. H. Cause of hen-feathering, 86.

Daniels, A. L., and Jordan, D. P. Wheat germ oil on fertility, and fat free rations, 185; Antirachitic properties of irradiated foods, and superheating, 453.

Davenport, H. A., and Ranson, S. W. Glycogen content of muscles in tetanus contracture, 466.

Dawson, J. A. Cannibalism in a ciliate, 335.

Day, L. A. See Adams, W. E., 163.

Dewey, E. T. See Green, R. G., 426.

Dieckmann, W. J. Hepatic lesion in eclampsia, 239.

Dixon, H. H., and Ranson, S. W. Ions on fatigue contracture, 165.

Doan, C. A. Precipitin tests with Anderson phosphatide fractions from tubercle bacilli, 672.

Dochez, A. R., Shibley, G. S., and Mills, K. C. Acute infection of respiratory tract, 562.

Dock, W., and Tainter, M. L. Circulatory changes after digitalis, 524.

Domm, L. V. Spermatogenesis following early ovariotomy, 338.

Dooley, M. S. See Koppanyi, T., 443.

Dragstedt, L. R., and Ellis, J. C. Total loss of gastric juice, 305; and Spurrier, B. Diversion of bile into vena cava and portal vein, 303; see Ellis, J. C., 304.

Drain, C. L. See Magee, C., 718.

Drury, D. R. See McMaster, P. D., 490.

Dunham, E. S. See Flowers, S. H., 572.

Duval, C. W. Urinary findings and renal pathology in streptococcal nephritis, 546.

Dye, J. A., and Maughan, G. H. Thyroidectomy and succinoxidase activity of surviving tissues, 441; Thyroidectomy and tissue respiration, 439.

Eaton, A. G. See Chidester, F. E., 141, 143.

Ecker, E. E., and Simon, M. A. Precipitin production with phosphorised caseinogen, 222; see Gross, P., 508.

Eckles, C. H. See Palmer, L. S., 58.

Eddy, C. E. See Geiling, E. M. K., 146.

Ekola, M. W. Subcutaneous tissue and sodium ricinoleate, 854.

Ellis, J. C., and Dragstedt, L. R. Liver autolysis in vivo, 304; see Dragstedt, L. R., 305.

Emery, F. E. See Griffith, F. R., Jr., 628.

Emmel, V. E. See Joranson, Y., 178.

Eneboe, J. B. See Hirschfelder, A. D., 258.

Erlanger, J. See Gasser, H. S., 647.

Ermatinger, L., Queen, F. B., and Parker, R. F. Skin bacteria after transplantation subcutaneously, 113.

Ernst, E. C. See Jorstad, L. H., 30.

Evanoff, M., and Sweany, H. C. Culturing bovine tubercle bacilli, 456.

Evans H. M., and Simpson, M. E. Impairment of birth mechanism by hormone of anterior hypophysis, 595; Hyperplasia of mammary apparatus by hypophyseal hormone, 597; Hyperplasia, adult virginal female mammary apparatus by hypophyseal hormone, 598; Stimulation of placentoma reaction in virgin by hypophyseal maturity hormone, 597.

Falk, I. S. See Stuppy, G. W., 314.

Fallon, M. See Stenstrom, W., 380.

Faust, E. C. Infection with sparganum stage of Oriental Diphyllobothrids, 252; Parasiticidal potencies of the methyl derivatives of rosanilin, 748; see Li, C., 250.

Feng, T. "Chilies" and digestive functions and metabolism, 273.

Field, J., II. See Martin, E. G., 292.
Fischer, A., and Parker, R. C. Differentiation and proliferation in vitro, 583; Technique for tissues in state of latent life in vitro, 585; see Parker, R. C., 580.
Fish, P. A. Blood phosphates in milk fever, 387.
Fishberg, E. H. Colloid osmotic pressure and concentration of serum proteins, 1.
Flesche, B. A., and McClendon, J. F. Determination of oxyhemoglobin by Duboscq colorimeter, 791.
Flexner, L. B., Berkson, J., Winters H., and Wolman, I. Antitryptic titre in pregnancy and in hyperthyroidism, 592.
Flinn, F. B. See Von Glahn, W. C., 200.
Flowers, S. H. Dunham, E. S., and Barbour, H. G. Addiction edema and withdrawal edema and morphine, 572.
Foster, P. C. Ineffectiveness of radiant energy on anemia, 751.
Foust, R., and Nelson, V. E. Protein intake, and reproduction, 521.
Francis, L. D., Smith, A. H., and Moise, T. S. Vitamins B and G, renal enlargement, with high concentrations of protein, 725.
Freund, J. Phagocytosis of red blood cells, and tannin, 876.
Fried, B. M. Phloridzin diabetes and vital staining, 809.
Friedman, M. H. Injections of urine from pregnant women, and ovary, 720.
Fries, M. E., and Lenz, M. Secondary rays from lipiodol and bismuth subnitrate on S. aureus, coli communis, 14.
Frobisher, M., Jr. Complement fixation test in yellow fever, 846 Scarlet fever streptococcus toxin, 50; see Rich, A. R., 397.
Fulmer, E. I., and Buchanan, R. E. Thermal increments and critical temperatures of biological reactions, 446.
Funk, C. Chemistry of oestrus producing hormone, 568; and Harrow, B. Male hormone, 325; Harrow, B., and Lejwa, A. Male hormone, 569.

Gage, I. M., and Herrmann, G. Experimental arteriogenous aneurysm, 760.
Garcia, T. See Katayama, I., 122.
Gardner, L. U. Differential cell count of peritoneal fluid, 690.
Gasser, H. S., and Erlanger, J. Action potential in fibers in spinal roots and somatic nerves, 647.
Gay, F. P., and Thompson R. Cultivate vaccine virus in growing embryo, 556.
Gay, H. H. Low alveolar oxygen and response to reflex stimulation, 826; Respiration and response to reflex stimulation, 828; Sodium sulphide and cyanide on reflex response, 825; Ventilation and response to reflex stimulation, 827.
Geiger, A. See Kligler, I. J., 229.
Geiger, J. C., and Meyer, K. F. Food poisoning with heat stabile paratyphoid poisons, 91.
Geiling, E. M. K., and Eddy, C. E. Hyperglycemic effect of vasopressin, oxytocin and pituitrin, 146; see Jensen, H., 219.
Gerard, J., and Broun, G. O. Test for bile salts in urine 209.
Gesell, R., and Nyboer, J. Synchronous experimental changes in respiratory and cardiac rhythms, 834.
Gettle, R. R., and Knoepp, L. F. Low oxygen and muscular contraction, 830.
Gibson, R. B. Latent tolerance in diabetics, 449.
Gies. W. J. Vitality of dental enamel, 69.
Gilding, H. P. See Rous, P., 497.
Gold, H., and Sussman, M. L. Pupillary reactions during anesthesia after morphine, 3.
Goltz, H. L. See Cori, C. F., 433.
Goss, H., and Schmidt, C. L. A. C and P balances during pregnancy and lactation, 104.
Gourley, M. F. Bacteria in non-tuberculous infections of urinary tract, 499.
Graham, C. E., and Griffith, W. H. Growth factors in liver, 715; see Griffith, W. H. 862.
Graham, H. T. Lactic acid increase in muscle under anesthetics, 641.
Gray, S. H., and Rabinovitch, J. KI and thyroid gland, 468; see Rabinovitch, J., 472.
Greeley, C. E. See Greeley, P. O., 828.
Greeley, P. O., and Greeley, C. E. Blood flow during periodic ventilation, 828.
Green, E. A. See Schultz, E. W., 97.
Green, R. G., and Wade, E. M. A natural infection by B. tularense, 626; Wade, E. M., and Kelly, W. Experimental tularemia, 260; Wade, E. M., and Dewey, E. T. Experimental tularemia, 426.
Greenberg, D. S. See Nice, L. B., 136.
Greenberg, S. L. See Nice, L. B., 136.
Greenwald, I. Urine pentose in normal metabolism, 321.
Griffith, F. R., Jr., and Emery, F. E. Adrenalin and pituitrin on volume of liver, 628.
Griffith, W. H. Growth on diet containing sodium benzoate, 354, 858; and Graham, C. E. Liver extract on food consumption, utilization and growth, 862; see Graham, C. E., 715.
Gross, P. Duration of anticoagulant action of heparin in vivo, 383; Purification of bacterial toxins by adsorption, 696; and Ecker, E. E. Anticomplementary action of purified heparin, 508.
Gruber, C. M. Blood pressure affected by "Vasopressin," "Oxytocin," pituitary extract, 243; Arterial blood pressure in skeletal muscles, and epinephrine, 472.
Gruehl, H. L. See Ratner, B., 8, 127, 197, 327, 679.
Gunnison, J. B., and Meyer, K. F. Susceptibility to Botulinum toxin type B, C, D, 89; see Meyer, K. F., 88.
Gunstad, B. See Harris, J. A., 851.
Guyer, M. F., and Claus, P. E. Foreign protein and insulin after ox-gall, 65.

Haagen, E. See Rivers, T. M., 494.
Hagebush, O. E. See Kinsella, R. A., 642, 857.
Hall, I. C. Maternally transmitted immunity to B. sordellii, 799.
Hall, V. E. See Martin, E. G., 292.
Halsey, J. T. Ephedrin on intestine and bronchi, 16.
Hamre, C., and Nichols, M. S. Exophthalmia in trout-fry, 63.
Harmon, P. H. See Dack, G. M., 49.
Harnes, A. R. Inverse relation between inorganic and lipoid phosphorus in blood, 360.
Harris, E. S. See Blinks, L. R., 836; see Osterhout, W. J. V., 124, 838.
Harris, J. A., Wilder, M. A., and Gunstad, B. Change of sex on intensity of heredity, 851.
Harris, W. H., and Larimore, O. M. Bacteriophage, in vivo prepared toxic substance of B. typhosus, 752; see Larimore, O. M., 753.
Harrow, B. See Funk, C., 325, 569.
Harvey, E. N. Stability of luminous substances of luminous animals, 133.
Hastings, A. B. See Shock, N. W., 780.
Haterius, H. O., and Nelson, W. O. Ovariectomy and transplantation in pregnancy, 659; and Pffiffner, J. J. Inhibition of oes-

trus by extracts of corpus luteum, 818; Pffiffner, J. J., and Nelson, W. O. Oestrus inducing effect of taurocholate, 820. see Herren, R. Y., 657; see Nelson, W. O., 660.
Hawkins, J. A. Effect of drying on virus group, 479; Resistance of causative agent of chicken tumor to organic solvents compared with vaccine virus and Herpes febrilis virus, 480.
Heidelberger, M., and Kendall, F. E. Reaction between proteins and diazotized aromatic amines in neutral solution, 482.
Heinbecker, P. Unmyelinated fibers of nerve, 349; and Bishop, G. H. Differentiation between fibers in involuntary nervous system, 645; see Bishop, G. H., 241.
Helff, O. M. Properties of Na, K, Mg, and Ca ions on duration of life, 797; and Clausen, H. J. Atrophy of anuran tail muscle during metamorphosis, 650.
Henriksen, E. See Chen, K. K., 348.
Henry, B. S. Spontaneous and forced dissociation of Br. abortus, 101.
Herber, E. C. See Slifer, E. H., 605.
Herren, R. Y., and Haterius, H. O. Oestrus and Achilles reflex time, 657.
Herrick, J., and Sheard, C. Irradiation of crude coal tar by quartz mercury lamps, 33.
Herrmann, G. R. Heart of race horse, 549; see Gage, I. M., 760.
Herrmann, L. G., and Cutler, E. C. Experimental production of chronic abscess of lung, 28.
Hess, A. F., Weinstock, M., and Rivkin, H. Increase in serum calcium by irradiated ergosterol, 199; Parathyroids and action of irradiated ergosterol, 555.
Hetherington, D. C. Production of epithelioid cells in lymph glands by non-tuberculous substances, 333.
Hetler, D. M., and Bronfenbrenner, J. Particulate size of bacteriophage, 644.
Higgins, G. M. See Sheard, C., 615
Hill, P. S. See Macht, D. I., 871.
Hill, S. E. Transportation of CO_2 by paraffine oil, 590.
Himwich, H. E., Chambers, W. H., Koskoff, Y. D., Nahum, L. H., and Adams, M. A. Hyperglycemia of diabetes, 120; and Nahum, L. H. Respiratory quotient of brain, 496.
Hines, H. M. See McClintock, J. T., 654.
Hinrichs, M. A. Ultraviolet radiation, stimulation and inhibition, 175.
Hirschfelder, A. D., Eneboe, J. B., and Parsons, R. L. Antagonism of magnesium sulphate by chlorides, 258; and Wright, H. N. Antiseptic activity of adsorbed protein, 787; and Wright, H. N. Is Schnitzer's "Interference" due to surface reactions, 789; see Wright, H. N., 790.
Hisaw, F. L., and Meyer, R. K. Oestrus hormone in urine, 586.
Hitz, J. B. See Leake, C. D., 288..
Hoag, L. A., and Marples, E. Dehydration of diarrhea with parenteral fluids, and acid-base status of blood, 374; Dehydration of diarrhea with parenteral fluids, and excretion of fixed acid and base, 376.
Hockett, A. J. Effect of alkalis on solubility of quinine salts, 613; Reciprocal activity of muscle coats of intestine, 813.
Hoskins F. M. See Snyder, F. F., 804.
Hou, H. See Lim, R. K. S., 270.
Hou, H. C. Behaviour of adrenal transplants in transparent chamber, 745.
Hu, C. H., Huie, C., and Lee, C. U. Slapping in transparent chamber, 745.
280; Reimann, H. A., and Kurotchkin, T. G. Filaments in siderotic nodules of spleen in splenomegaly, 413; and Lee, C. U. Feeding sandflies for experimental transmission of kala-azar, 277.
Hubbard, R. S. Reducing reaction of cerebrospinal fluid, 78; and Allison, C. B. Comparison of icteric index and direct Van den Bergh tests, 438; see Allison, C. B., 629.
Huggins, C. H. See Curtis, G. M., 70.
Huie, C. See Hu, C. H., 280.
Hull, T. G. See Arnold, L., 159.
Husted, H. G. See Bogert, M. T., 721.

Ingram, W. R. Metamorphosis by injection of inorganic iodine, 191.
Irwin, M. Penetration of alkaloids into vacuoles of living cells, 135; Predicting penetration of dyes into living cells, 125.
Ivy, A. C., Lueth, H. C., and Kloster, G. A vaso-depressor phenomenon caused by some cholecystokinin preparations, 309; see Lueth, H. C., 310; see Seitz, I. J., 463.

Jaffe, R. H., Willis, D., and Bachem, A. Electric currents on arteries, 166.
Jenkins, H. P. Experimental intestinal obstruction, 464.
Jennings, F. B., Jr. Dissociation of a hemolyticus, 145.
Jensen, H., Geiling, E. M. K., and Wintersteiner, O. Isolation of insulin in crystalline form, 219; see Chen, K. K., 378.
Jensen, J. Cardiovascular response to adrenalin subcutaneously, 259.
Johlin, J. M. Isoelectric point of gelatin, 702.
Johnson, A. F. See Sheard, C., 618.
Johnson, C. A. See Rappaport, B. Z., 771.
Johnson, C. C. Toxicity of nitroprusside, 102.
Jones, D. B. See Phillips, M., 320.
Jones, R. Erythrocyte sedimentation rate of citrated blood, and plasma proteins, 115.
Joranson, Y., Emmel, V. E., and Pilka, H. J. Arterial supply of testis, 178.
Jordan, D. P. See Daniels, A. L., 185, 453.
Jordan, E. O. See Dack, G. M. 301.
Jorstad, L. H., and Ernst, E. C. Dietary and X-rays, 30.
Julianelle, L. A., and Avery, O. T. Intracutaneous vaccination with pneumococcus, antibody response, 224; Intracutaneous vaccination with pneumococcus, hypersensitiveness, 227; Intracutaneous vaccination with pneumococcus, resistance to infection, 226.
Jung, F. T., and Skillen, W. F. Thyroparathyroidectomy and teeth, 598.

Kammer, A. G. See Leake, C. D. 288.
Karelitz, S., Cohen, P., and Leader, S. D. Inhibition of insulin activity, 11.
Katayama, I., and Garcia, T. Blood sugar during glycolysis, 122.
Keefer, C. S. See Berglund, H., 418.
Kelly, W. See Green, R. G., 260.
Keltch A. K. See Mudd, S., 392.
Kendall, F. E. See Heidelberger, M., 482.
Kennedy, C. See Palmer, L. S., 427.
Key, J. A. Pathogenic properties of organisms in arthritis, 863.
Kik, M. C. See Sure, B., 603.
Kilborn, R. B., Pierce, H. B. and Tittsler, R. P. Bactericidal action of indole, 391.
Kinsella, R. A., and Hagebusch, O. E. Experimental arthritis and allergy, 642; Experimental streptococcus arthritis, and sodium salicylate on allergy, 857.
Kleitman, N. Morphine vs. feeding in conditioned salivary response, 167; and Palmer, D. Spontaneous extinction of morphine salivary conditioned response, 168.
Kligler, I. J., and Geiger, A. Lactic acid con-

tent of blood in trypanosome infection, 229; Muckenfuss, R. S., and Rivers, T. M. Transmission of fowl-pox by mosquitoes, 128.
Kloster, G. See Ivy, A. C., 309.
Knoepp, L. F. See Gettle, R. R., 830.
Knowlton, G. C. See Stearns, G., 181.
Koch, R. See Semsroth, K., 516.
Koehler, A. E. Dual calorogenic activity of suprarenal gland, 296.
Koppanyi, T. Pilocarpine on pupil, 79; Sympathomimetic drugs on pupil, 80; Compensatory eye positions in complete Torticollis, 81; and Dooley, M. S. Response of kidney and spleen to epinephrin, 443.
Koskoff, Y. D. See Himwich, H. E., 120.
Krasnow, F., and Rosen, A. S. Determination of lipin phosphorus, 67.
Krueger, A. P., and Schultz, E. W. Ultrafiltration studies on virus of poliomyelitis, 600; and Tamada, H. T. Ultrafiltration studies on bacteriophage, 530; see Schultz, E. W., 100.
Kugelmass, I. N. Tertiary dissociation constant of phosphoric acid, 129.
Kunde, M. M., and Neville, M. Cutaneous reflex in experimental hypothyroidism, 776.
Kurotchkin, T. G. See Hu, C. H., 413; see Reimann, H. A.. 410.

Lamson, R. W. Epinephrin and skin reactions to pollen and to histamine, 612; Skin reactions to pollen and to histamine, 611.
Landsteiner, K. Hemagglutination by tumor extracts, 134.
Lanford, J. A. In vitro cultivation of spirochete, 761.
Larimore, O. M., and Harris, W. H. Bacteriophage in experimental typhoid peritonitis, 753; see Harris, W. H., 752.
Larson, E., and Alkourie, L. E. Tetany and blood calcium after thyroparathyroidectomy, 210.
Laurens, H., and Mayerson, H. S. Ineffectiveness of radiant energy on hemorrhagic anemia, 750.
Lawrence, J. S., Tompkins, E. H., and Cunningham, R. S. Production of monocytes and epithelioid cells in tissues by injection of irritants, 331.
Leach, H. See Macht, D. I., 330.
Leader, S. D. See Karelitz, S., 11.
Leake, C. D., Kammer, A. G., and Hitz, J. B. Afferent nerve fibers in meningeal blood vessel walls, 288; see Millzner, R. J., 526.
Leavitt, H. L. See Thornton, R. C. B., 811.
Lee, C. U. See Hu, C. H., 277, 280.
Lee, M. O. See Cunningham, B., 221.
Lejwa, A. See Funk, C., 569.
Lennox, W. F. See Weiss, S., 706.
Lenz, M. See Fries, M. E., 14.
Lewis, H. B. Cystine in sweat of cystinurics, 69.
Li, C., and Faust, E. C. Infection of cyclops with coracidium of Oriental Diphyllobothrids, 250.
Li, C. P. See Wu, H., 737.
Liddell, H. S., and Anderson, O. D. Conditioned reflexes, 81.
Leiter, L. Experimental edema, 173.
Lieb, C. C., and Mulinos, M. G. Sodium isoethyl-barbituric as laboratory anesthetic, 708.
Lieb, C. W., and Tolstoi, E. Effect of meat diet on chemical constituents of blood, 324.
Lillie, R. S. Circuit transmission and interference of activation waves in passive iron wire, 316.
Lim, R. K. S., and Hou, H. Mechanical factors and "basal" gastric secretion, 270; Denervated spleen in adrenalectomy, 271.
Lindeman, V. F. Chemicals and heart rhythm, 816; Tail skin histolysis during metamorphosis, 451.
Little, M. E. Transplantation of mammalian tissues, 372.
Lloyd, W. Action of calcium on vertebrate heart, 506.
Loeb, L., and Bassett, R. B. Hormones of anterior pituitary and thyroid, 860; and Lorberblatt, I. Metals combined with urease or coenzymes of amoebocytes, 111.
Loebel, R. O., and Shorr, E. Respiratory depressants on respiratory quotients of excised tissue, 722.
Loehwing, W. F. Mineral nutrient balance related to sap hydrion concentration, 189; Isolation on mineral nutrition, 662.
Long, P. H., and Olitsky, P. K. Resistance of streptococci and poliomyelitic virus in glycerol, 337.
Lorberblatt, I. See Loeb, L., 111.
Luck, J. M., and Spaulding, J. Insulin on protein metabolism, 631.
Lueth, H. C., Orndoff, B. H., and Ivy, A. C. Histamine and gall bladder evacuation, 310; see Ivy, A. C., 309.

McAmis, A. J., Anderson, W. E., and Mendel, L. B. Iodine values of rat fat, 515.
McCallum, J. T. C. See Zerfas, L. G., 399,
McCartney, J. L. Male hormone, 686.
McClelland, P. H. See Cannon, P. R., 157.
McClendon, J. F. Variations in basal metabolism and skin temperature, 784; and Burr, G. Basal metabolism and ovarian hormone, 782; Burr, G., and Conklin, C. Basal metabolism and injection of follicular hormone, 265; Burr, G., and Wildebush, F. F. Induction of menstruation by ovarian hormone, 430; and Sanford, J. R. Iodine in relation to goiter, 263; see Flesche, B. A., 791; see Wildebush, F. F., 785.
McClintock, J. T., and Hines, H. M. Heat labile toxins in closed intestinal loops, 654.
McClure, G. pH of blood and sodium cyanide, 829.
McCordock, H. A. Thyroid changes following KI and anterior pituitary, 109.
McCurdy, H. M. See Witschi, E., 655.
McJunkin, F. A. Monocytic response to peroxydase test, 55; Stimulation and phagocytic activity of vascular endothelium, 294.
McKinley, E. B. Chloroform-treated tissue-vaccine in experimental herpetic encephalitis, 24; Experimental herpetic encephalitis with herpes virus, 21; Experimental immunization with herpes virus, 19; Experimental rinderpest, 22; Leptospira icterohaemorrhagiae, 26; Experimental herpetic encephalitis through respiratory tract, 699; Salivary gland poison of Aedes aegypti, 806.
McMaster, P. D., and Drury, D. R. Source of fibrinogen, 490.
Ma, W. C. See Pillat, A., 739.
MacDowell, E. C. See Richter, M. N., 362.
Macht, D. I., and Hill, P. S. Urethane and intestinal peristalsis, 871 and Leach, H. Methyl and ethyl alcohol mixtures, and behaviour, 330; see Olmsted, J. M. D., 533.
Magee, C., Drain, C. L., and Boyd, J. F. Dental caries and mouth acidity, 718.
Maltaner, E. Sensitizing effect of cholesterol for antigen, and ergosterol, 677.
Mammoser, L. F., and Boyd, T. E. Chemical agents and absorption of histamine from intestine, 765.

Manwaring, W. H., Azevedo, J. L., and Boone, T. H. Protein denaturization rate and specific antibodies, 525.

Marine, D., Baumann, E. J., and Cipra, A. Goiter produced by vegetables, 822.

Marples, E. See Hoag, L. A., 374, 376.

Martin, E. C., Field, J. II, and Hall, V. E. Tissue and blood lactates, and muscular exercise, 292.

Master A. M. Changes in hypertension roentgenogram and electrocardiogram, 367.

Mattill, H. A., and Pratt, E. D. Tea as source of vitamin C, 82.

Matzger, E. Relationship of atopens as indicated by passive transfer studies, 634.

Maughan, G. H. See Dye, J. A., 439, 441.

Mayerson, H. S. See Laurens, H., 750.

Megrail, E. Filtration of virus of Avian molluscum, 217.

Mehrtens, H. G., and Pouppirt, P. S. Hyperpyrexia and permeability of meninges, 286.

Meleney, F. L. Aerobic and anaerobic examples of hemolytic bacterial synergism, 205.

Mendel, L. B., and Vickery, H. B. An attempt to secure "refection," 552; see McAmis, A. J., 515.

Menville, L. J., Blackberg, S. N., and Ane, J. N. Motility of gastro-intestinal tract in rickets, 758.

Meyer, K. F., and Gunnison, J. B. Cl. botulinum type D Sp. N., 88; see Gunnison, J. B., 89; see Geiger, J. C., 91.

Meyer, R. K. See Hisaw, F. L., 586.

Miles, W. R. Duration of sleep and insensible perspiration, 577.

Miller, L. See Mitchell, H. S., 835.

Mills, K. C. See Dochez, A. R., 562.

Millzner, R. J., and Leake, C. D. Toxicity of "Eucupin" in local analgesia, 526.

Minot, A. S., and Cutler, J. T. Guanidine retention, calcium reserve carbon tetrachloride and chloroform poisoning, 138; Increase in guanidine-like substances in acute liver injury and eclampsia, 607.

Mitchell, H. S., and Miller, L. Spinach in anemia, 835.

Moise, T. S., and Smith, A. H. Pathogenesis of pulmonary suppuration, 723; see Francis, L. D., 725.

Moore, L. M. Hypothermia due to localized lesions in midbrain, 48; Body temperature regulation and labyrinth destruction, 104; Body temperature regulation, and lesions of vestibular nuclei, 106.

Morgan, A. F., and Smith, L. L. W. Development of Vitamin A during ripening of tomatoes, 45.

Moritz, A. R. Cholesterol, ultra-violet irradiation on growth of tubercle bacilli in vitro, 43.

Morrell, J. A., Powers, H. H., and Varley, J. R. Action of follicular hormone, 685.

Moses, M. J. See Beard, S. D., 13.

Mu, J. Neoarsphenamin and blood platelets, 407.

Muckenfuss, R. S. See Kligler, I. J., 128; see Rivers, T. M., 494.

Mudd, E. H. H., Mudd, S., and Keltch, A. K. Echinid egg-waters and surface potential difference of sperm, 392.

Mudd, S. See Mudd, E. B. H., 392.

Mulinos, M. G. See Lieb, C. C., 708.

Muller, E. F., and Petersen, W. F. Lymph production and heat regulation, 169.

Murlin, J. R. See Collens, W. S., 485.

Myers, V. C. See Beard, H. H., 510.

Nahum, L. H. See Himwich, H. E., 120, 496.

Nelson, V. E. See Foust, R., 521; see Taylor, M. W., 521.

Nelson, W. C. See Baldwin, W. M., 588.

Nelson, W. O., and Haterius, H. O. Reaction of ovariectomized pregnant rats to ovarian grafts, 660; see Haterius, H. O., 659, 820.

Neville, M. See Kunde, M. M., 776.

Nibler, C. W., and Turner, C. W. Ovarian hormone content of urine in pregnancy, 882.

Nice, L. B., Greenberg, D. S., and Greenberg, S. L. Adrenalin and fatiguability of muscle, 136.

Nicholas, J. S. Movements in transplanted limbs, 729; Transplantation of tissues, 731.

Nichols, M. S. See Hamre, C., 63.

Nicholls, E. E. See Cecil, R. L., 6.

Nicholson, H. Oxygen and CO_2 on flow of cerebro-spinal fluid, 834.

Nicholson, T. F. Bacterial synergism on endo medium, 41.

Nungester, W. J. Determining hardness of nutrient agar, 457.

Nurnberger, C. E. See Stenstrom, W., 380.

Nyboer, J. See Gesell, R., 834.

Okey, R. Determination of cholesterol as digitonid, 518.

Olitsky, P. K. See Long, P. H., 337.

Olmsted, J. M. D., and Mackler, H. Phosphocreatine and insulin, 533.

Olmsted, W. H., and Whitaker, W. Determination of volatile fatty acids, 108.

Oppenheimer, B. S. See Ralli, E. P., 9.

Orndoff, B. H. See Lueth, H. C., 310.

Osgood, H. S. See Pierce, H. B., 347.

Osterhout, W. J. V. Penetration of electrolytes, 192; and Harris, E. S. Bioelectrical aspects of all or none law, 838; and Harris, E. S. Reversible changes in living protoplasm, 124; see Blinks, L. R., 836.

Otto, H. L. Altering position of heart affecting electrocardiogram, 204; Altering venous inflow to the heart, effect on electrocardiogram, 202; Barium chloride and ouabain upon ventricular fibrillation, 201.

Pacheco, G. A. See Curtis, G. M., 874.

Palmer, D. See Kleitman, N., 168.

Palmer, L. S., Eckles, C. H., and Schutte, D. J. Magnesium sulfate in retention of Ca and P., 58; and Kennedy, C. Growth, and fat in diet, 427.

Parker, G. H. Ciliary currents related to transportation of spermatozoa, 52.

Parker, R. C., and Fischer, A. Classification of "fibroblasts," 580; see Fischer, A., 583, 585.

Parker, R. F. See Ermatinger, L., 113.

Parsons, R. L. See Hirschfelder, A. D., 258.

Pearce, L., and Casey, A. E. Monocytic reaction in virus III disease, 666; Monocytic reaction in neoplasia, 668.

Pearl, R. Pathological relation between cancer and tuberculosis, 73.

Petersen, W. F. See Muller, E. F., 169.

Peterson, V. L. See West, E. S., 351.

Pffiffner, J. J. See Haterius H. O., 818, 820.

Phillips, M., Weihe, H., Jones, D. B., and Csonka, F. A. Demethoxylation of lignin, 320.

Phillips, R. S., and Robertson, D. F. Irradiated ergosterol on thrombocyte counts, 639.

Philpott, C. H. Effect of toxins and venoms on protozoa, 522.

Pierce, H. B., Osgood, H. S., and Polansky, J. B. Absorption of glucose from alimentary tract in vitamin B deficiency, 347; see Kilborn, R. B., 391.

Pilka, H. J. See Joranson, Y., 178.

Pillat, A., and Ma, W. C. Columnar epithelium of ciliary body, 739.

Pohlman, A. G. Apparatus for quantitative testing of air and bone transmitted speech, 355.
Polansky, J. B. See Pierce, H. B., 347.
Pomerene, E. See Beard, H. H., 328.
Pouppirt, P. S. See Mehrtens, H. G., 286.
Powers, H. H. See Morrell, J. A., 685.
Pratt, A. D. See Mattill, H. A., 82.

Queen, F. B. See Armatinger, L., 113.
Quigley, J. P. Insulin on gastric motility, 769.

Rabinovitch, J. Intravenous injection of ox and dog serum, causing death, 117; and Gray, S. H. Infections and thyroid gland, 472; see Gray, S. H., 468.
Ralli, E. P., and Oppenheimer, B. S. Peripheral circulation in "tobacco angina," 9.
Rane, L. Virulence, electrophoresis and conversion characteristics of B. phaseoli sojense, S. and R., 299.
Ranson, S. W. See Davenport, H. A., 466; see Dixon, H. H., 165.
Rappaport, B. Z., and Johnson, C. A. Fractionation of pollen proteins, and skin reactions, 771.
Rasmussen, A. T. Cell types in pars anterior of adult male, 424.
Ratner, B., and Gruehl, H. L. Respiratory anaphylaxis, following sensitization, 127; Transmission of respiratory anaphylaxis from mother to offspring, 8; Methods in study of anaphylaxis, 197; Sensitizing dose in respiratory anaphylaxis, 327; Congenital hypersensitiveness transmitted to the third generation, 679.
Rawlins, A. L. See Brown, J. B., 704.
Reimann, H. A., Kurotchkin, T. G., and Tso, E. Etiology of splenomegaly, 410; and Sia, R. H. P. Kusama's "Typhus Bacillus immune horse serum" in experimental typhus fever, 267; see Hu, C. H., 413.
Reuterskiold, K., and Andrews, E. Post operative blood chemical studies, 17.
Rhoads, C. P. See Stewart, F. W., 664.
Rich, A. R., Bumstead, J. H., and Frobisher, M., Jr. Haemorrhagic glomerular lesions produced by filtrates of viridans cultures, 397.
Richter M. N., and MacDowell, E. C. Experimental transmission of leukemia, 362.
Rivers, T. M., Haagen, E., and Muckenfuss, R. S. Virus infection and virus immunity in tissue cultures, 494; see Kligler, I. J., 128.
Rivkin, H. See Hess, A. F., 199, 555.
Robb, G. P. See Weiss, S., 706.
Robertson, D. P. See Phillips, R. A., 639.
Ronzoni, E. Amylase on formation of hexosephosphate by muscle extract, 356.
Rose, M. I. See Cook, S. F., 529.
Rose, M. S., Vahlteich, E. M., and Bloomfield, E. L. Whole wheat and hemoglobin regeneration, 322.
Rosen, A. S. See Krasnow, F., 67.
Ross, V. Oral immunization with disrupted, and avirulent pneumococci, 565.
Rous, P., and Gilding, H. P. Meaning of Bier's spots, 497.
Rupp, A., and Schlutz, F. W. Motility of stomach in asthenia, 621.

Sabin, A. B. Agglutination test, typing of pneumococci, and determination of antibody, 492.
Sah, P. P. T. See Wu, H., 737.
Salant, W. Calcium and potassium on ephedrine, 680; and Washeim, H., Jr. Calcium and action of pilocarpine and atropine, 512.
Sandels, M. R. See Sherman, H. C., 536.
Sanford, J. R. See McClendon, J. F., 263.
Scharles, F. H. See West, E. S., 351.
Schlutz, F. W., and Wetzel, W. W. Ultraviolet absorption and immunity, 878; See Rupp, A., 621; see Wilder, R. L., 624.
Schmidt, C. L. A. See Goss, H., 104.
Schultz, E. W. Infection with poliomyelitis virus by gastro-intestinal route, 632; and Green, E. A. Trypsin susceptible bacteriophage adapted to trypsin, 97; and Krueger, A. P. Inactivation of Staphylococcus bacteriophage by methylene blue, 100; see Krueger, A. P., 600.
Schutte, D. J. See Palmer, L. S., 58.
Schwartzbach, S., and Uhlenhuth, E. Anterior lobe substance, thyroid stimulator, feeding effect, 151; Anterior lobe substance in absence of thyroid gland, 153; Anterior lobe, thyroid stimulator, and basal metabolism, 389; see Uhlenhuth, E., 149; see Uhlenhuth, E., 152.
Scott, J. P. See Zerfas, L. G., 399.
Seitz, I. J., and Ivy, A. C. Effects of pancreatectomy, 463.
Semsroth, K., and Koch, R. Pathogenesis of endocarditis, 516.
Sheard, C., and Higgins, G. M. Ultraviolet light, solar irradiation and cod-liver oil on fertility and incubation, 615; and Johnson, A. F. Potentiometric and spectrophotometric changes by irradiation, 618; see Herrick, J., 33; see Taylor, N. W., 257.
Sherman, H. C., and Sandels, M. R. Heat-stable factor of Vitamin B group (factor P-P, Vitamin B_2 or G), 536.
Sherman, J. M. See Stark, C. N., 343.
Shipley, G. S. See Dochez, A. R., 562.
Shipley, R. A. See Beard, H. H., 328.
Shock, N. W., and Hastings, A. B. Determination of acid-base balance of blood, 780.
Shonle, J. A. See Zerfas, L. G., 399.
Shope, R. E. Cholesterol in blood, 336.
Shorr, E. See Loebel, R. O., 722.
Shwartzman, G. Immune sera and reactivity to B. typhosus filtrates, 131; Local skin reactivity to culture filtrates of microorganisms, 207; Preparation of B. typhosus toxic substances for local skin reactivity, 843.
Sia, R. H. P. Antipneumococcus protective substances in normal serum, 284; see Reimann, H. A., 267.
Siebert, W. J. Auto and homoiotransplantation of thyroid gland into brain, 236; Effect of graded degrees of heat upon cartilage in transplantation, 238.
Simmers, M. H. X-Ray diffraction spectra of Glycine, 527.
Simon, M. A. See Ecker, E. E., 222.
Simpson, M. E. See Evans, H. M., 595, 597, 598.
Sinclair, R. G. Diet and phospholipid fatty acids, 793; Inanition and phospholipid fatty acids, 795; Influence of food fat on constitution of phospholipids, 436.
Skillen, W. G. See Jung, F. T., 598.
Slifer, E. H., Herber, E. C., Blumenthal, R., Sun, T. P. and Wang C. C. Specific conductivity of protozoan cultures, 605.
Smith, A. H. See Francis, L. D., 725; see Moise, T. S., 723.
Smith, L. L. W. See Morgan, A. F., 45.
Smith, M., and Campbell, J. R., Jr. Lymphatic drainage of joint cavities, 395.
Smith, M. E. See Sure, B., 72.
Snyder, F. F., and Hoskins, F. M. Concentration of amino-acids, urea and non-protein nitrogen in blood during pregnancy, 804.
Somogyi, M. Preparation of blood filtrates for analysis, 353.
Spaulding, J. See Luck, J. M., 631.
Spurrier, B. See Dragstedt, L. R., 303.
Stainsby, W. J. See Cecil, R. L., 6.

Stark, C. N., Sherman, J. M., and Stark, P. Destruction of botulinum toxin by B. subtilis, 343.
Stark, P. See Stark, C. N., 343.
Stearns, G., and Boyd, J. D. Calcium and phosphorus and hyperparathyroidism, 717; and Knowlton, G. C. Nitrogen metabolism of diabetic children, 181.
Stenstrom, W., Watkins, C. H., Nurnberger, C. E., and Fallon, M. Influence of cathode rays on leucocytes 380.
Stewart, F. W., and Rhoads, C. P. Lesions in nasal mucous membrane in poliomyelitis, 664.
Still, K. S. Stomach activity during jaundice, 770.
Streicher, M. H. Experimental uremia, uremic enteritis, 171.
Stuppy, G. W., Falk, I. S., and Cannon, P. R. Local immunity in lungs immunized against pneumococci, 314.
Sun, T. P. See Slifer, E. H., 605.
Sure, B., and Kik, M. C. Influence of maternal diet on hemoglobin concentration in nursing young, 603; and Smith, M. E. Hypoglycemia in nursing young with vitamin B deficiency, 72.
Sussman, M. L. See Gold, H., 3.
Swanson, E. E. See Zerfas, L. G., 399.
Sweany, H. C. See Evanoff, M., 456.
Szurak, S. A. Calcium gluconate as therapeutic agent, 773.

Tainter, M. L. See Dock, W., 524.
Tamada, H. T. See Krueger, A. P., 530.
Tanner, F. W., and Bollas, G. D. Disinfectants and yeasts, 154.
Taylor, A. B. See Wenner, W. F., 188.
Taylor, M. W., and Nelson, V. E. Sources of Vitamin E., 521.
Taylor, N. W., and Sheard, C. Microscopic and X-Ray Investigations on calcification of tissue, 257.
Thienes, C. H. Ergot alkaloids on intestine and uterus, 501; Ephedrine-epinephrine antagonism, 500; Nicotine tolerance, 845.
Thompson, C. P. See Chidester, F. E., 143.
Thompson, R. Vaccinal encephalitis and testicular virus, 559; see Gay, F. P., 556.
Thompson, W. O., and Alper, J. M. Posture, and velocity of blood flow, 697.
Thornton, R. C. B., and Leavitt, H. L. Locke's vs. Tyrode's for perfusion of segments of intestine, 811.
Tittsler, R. P. See Kilborn, R. B., 391.
Tolstoi, E. See Lieb, C. W., 324.
Tompkins, E. H. See Lawrence, J. S., 331.
Tripoli, C. J. Preparation of culture media of feces for pathogenic amebas, 245.
Tscherkes, L. A. Anemia treated by liver, and growth, 869.
Tso, E. Growth and reproduction on vitamin C free diet, 276; see Reimann, H. A., 410.
Tunnicliff, R. Use of paramecia for studying toxins and antitoxins, 213.
Turner, C. W. See Nibler, C. W., 882.
Turner, R. G. Acute infections in animals with inadequate supply of Vitamin A, 233.
Turner, R. H. Oxidation of glutathione in human erythrocytes, 541.
Twitty, V. C. Correlation in development of structures associated with transplanted eyes, 726.

Uhlenhuth, E., and Schwartzbach, S. Anterior lobe substance, thyroid stimulator, and precocious metamorphosis, 149; Effect of anterior lobe substance on thyroid gland, 152; see Schwartzbach, S. S., 151, 153, 389.
Vahlteich, E. M. See Rose, M. S., 322.
VanAllen, C. M. See Adams, W. E., 163.
Van Campenhout, E. Origin and development of sympathetic nervous system, 824.
Van der Jagt, E. R. Histolytic influence of atrophying gills during metamorphosis, 183.
Varley, J. R. See Morrell, J. A., 685.
Vickery, H. B. See Mendel, L. B., 552.
Von Glahn, W. C., and Flinn, F. B. Chemical and pathological effect of copper on liver, 200.

Waddell, M. C. Reflex arcs and degeneration of extrinsic nerves, 867.
Wade, E. M. See Green, R. G., 260, 426, 626.
Walsh, E. L. See Crandall, L. A. 307.
Wang, C. C. See Slifer, E. H., 605.
Washeim, H., Jr. See Salant, W., 512.
Watkins, C. H. See Stenstrom, W., 380.
Weihe, H. See Phillips, M., 320.
Weil, A. Lipin content of nervous system in ether anesthesia, 776.
Weinstock, M. See Hess, A. F., 199, 555.
Weiss, S., Lennox, W. F., and Robb, G. P. Dilator effect of histamine on cerebral vessels, 706.
Wendel, W. B. Oxidation of lactic acid by erythrocytes, 865.
Wenner, W. F., and Blanchard, E. W. Intravenous injections of alkali on action of curare, 182; and Taylor, A. B. Changes in ion concentration of blood upon reflex time, 188.
West, E. S., Peterson, V. L., and Scharles, F. H. Estimation of true blood sugar, 351.
Wetzel, W. W. See Schlutz, F. W., 878.
Whitaker, W. See Olmsted, W. H., 108.
White, B. See Bunney, W. E., 801.
Wildebush, F. F., and McClendon, J. F. Assay ovarian hormone in blood and urine, and menstrual cycle, 785; see McClendon, J. F., 430.
Wilder, M. A. See Harris, J. A., 851.
Wilder, R. L., and Schlutz, F. W. Volatile fatty acids on gastric motor mechanism, 624.
Williams, O. B. Heat resistance of B. subtilis spores, 95.
Willis, D. See Jaffe, R. H., 166.
Winsor, A. L. See Bayne, T. L., 342.
Winters, E. S. See Bayne, T. L., 342.
Winters, H. See Flexner, L. B., 592.
Wintersteiner, O. See Jensen, H., 219.
Wintrobe, M. M. Hemoglobin standards, 848; Urea concentration test, 40.
Witschi, E., and McCurdy, H. M. Freemartin effect in experimental parabiotic twins, 655.
Wolf, O. M. Transplants of pituitary and reproduction, 692.
Wolman, I. See Flexner, L. B., 592.
Wood, W. L. See Dack, G. M., 301.
Woodruff, L. L. Thirteen thousand generations of paramecium, 707.
Wright, H. N., and Hirschfelder, A. D. Colloidal state of antiseptics and arsenicals, 790; see Hirschfelder, A. D., 787, 789.
Wu, C. K. See Chen, K. K., 348.
Wu, H. Concentrating protein solutions without denaturation, 744; Preparation of globin, 741; Sah, P. P. T., and Li, C. P. Composition of antigen-precipitin precipitate, 737.

Yang, C. S., and Berglund, H. Reticulocyte behavior in anemias from malaria and from pernicious anemia, 417; see Berglund, H., 418.
Yeager, J. F. Pyridine test for chloroform, 403.

Zerfas, L. F., McCallum, J. T. C., Shonle, H. A., Scott, J. P., Swanson, E. E., and Clowes, G. H. A. Induction of anesthesia by sodium iso-amyl-ethyl barbiturate, 399.

SUBJECT INDEX

(*The numerals indicate the page.*)

Abscess, lung, and intratracheal inoculation, 163.
  lung, chronic, production of, 28.
Achlorhydria during fever, and histamine test, 4-2.
Acid and base excretion, fixed, 376.
  and gastric motor mechanism, 624.
  and dental caries, 718.
Acid-base balance of blood, determination of, 780.
  status of blood, and parenteral fluids, 374.
Aconitine, effects of, 221.
Action potential in fibers in spinal roots and somatic nerves, 646.
Adrenal transplants in transparent chamber, 745.
Adrenalin and fatiguability of muscle, 136.
  cardiovascular response, 259.
  and pituitrin, and liver, 628.
Adrenalectomy, denervated spleen in, 271.
Aedes aegypti, salivary gland poison of, 806.
Agar, hardness of nutrient, 457.
Agglutination test, typing pneumococci, determination of antibody, 492.
Alcohol, ethyl and methyl, on behaviour, 330.
Alkali injections and curare, 182.
  and solubility of quinine salts, 613.
Alkaloids from Corydalis ambigua, 735.
  penetration of, 135.
Allergy, and experimental arthritis, 642.
  relationship of atopens by passive transfer studies, 634.
  and transmission of hypersensitiveness, 679.
  B. typhosus toxic substances for skin reactivity, 843.
  fractionation of pollen proteins, and skin reactions, 771.
  skin, and sodium salicylate, 857.
Amebas, pathogenic, preparation of culture media, 245.
Amines, diazotized aromatic, and proteins in neutral solution, 482.
Amylase and formation of hexose-phosphate by muscle extract, 356.
Amytal as laboratory anesthetic, 708.
Analgesia, toxicity of "Eucupin" in, 526.
Anaphylaxis, respiratory, transmission of, 8.
  respiratory, 127.
  methods in study of, 197.
  respiratory, sensitizing dose, 325.
Anastomosis, uretero-ureteral, 18.
Anemia, and radiant energy, 751.
  and spinach, 835.
  blood regeneration in, 510.
  deficiency, and cod liver oil, 418.
  from malaria, and pernicious anemia, 417.
  hemorrhagic, and radiant energy, 750.
  treated by liver, and basal metabolism, 872.
  treated by liver, and growth, 869.
Anesthesia after morphine, pupillary reactions, 3.
  induced by sodium iso-amyl-ethyl barbiturate, 399.
  lipin content of nervous system in, 776.
  effect of magnesium sulphate, antagonized by KCl, RCl, NaCl, 258.
  and lactic acid in muscle, 641.
Aneurysm, experimental arteriovenous, 760.
"Angina, tobacco" and peripheral circulation, 9.
Antagonism of magnesium sulphate, KCl, RCl, NaCl, 258.
  properties of Na, K, Mg, and Ca ions on duration of life, 797.
Antibody response to intracutaneous vaccination with pneumococcus, 224.
  determination, agglutination, typing of pneumococci, 492.
  specific, and protein denaturization rate, 525.
Antigen-precipitin precipitate, 737.
Antiseptic activity of adsorbed protein, 787.
  and arsenicals, colloidal state of, 790.
Arrhythmias, experimental cardiac, 570.
Arsenic and Vitamin A-free diet with iron iodide, 143.
  ferrous sulphate and copper sulphate, and Vitamin A-free diet with iron, 141.
  and antiseptics, colloidal state of, 790.
Arsphenamin-neo, and blood platelets, 407.
Arteries supply of testis, 178.
  and electric currents, 166.
Arthritis, and sodium salicylate on skin allergy, 857.
  bacteriology of blood, 6.
  experimental, 642.
  pathogenic properties of organisms in, 863.
Ascites, control of, 70.
Asthenia and stomach motility, 621.
Atopens, relationship indicated by passive transfer studies, 634.
Atrophy during metamorphosis, 650.
Atropine and pilocarpine, effect of calcium on, 512.
Avian molluscum, filtration of virus, 217.

Bacilli, meta-dysentery, 545.
  paratyphoid, experimental food poisoning with, 301.
  tubercle, 456.
Bacillus coli communis and aureus, radiation on, 14.
  sordellii, maternally transmitted immunity to, 799.
  subtilis, destroying Botulinum toxin, 343.
  spores, heat resistance, 95.
  typhosus filtrates, and immune sera, 131.
  typhosus toxic substances for local skin reactivity, 843.
  toxic substance of, and bacteriophage, 752.
  typhus immune horse serum, 267.
Bacteria and acute respiratory tract infection, 562.
  complement fixation test in yellow fever, 846.
  gram structure of cocci, 364.
  in non-tuberculous infections of urinary tract, 499.
  skin after transplantation, 113.
  synergism, hemolytic, 205.

  synergism on endo medium, 41.
Bactericidal action of indole, 391.
Bacteriology, composition of antigen-precipitin precipitate, 737.
  of blood in arthritis, 6.
Bacteriophage in experimental typhoid peritonitis, 753.
  particulate size, 644.
  staphylococcus, inactivated by methylene blue, 100.
  trypsin susceptible, adapted to action of trypsin, 97.
  ultrafiltration studies on, 530.
  upon in vivo prepared substance of B. typhosus, 752.
Barium chloride and ouabain, and ventricular fibrillation, 201.
Bartonella infection and ectoparasites, 157.
Basophilia and resistance, 670.
Behaviour, and ethyl and methyl alcohol, 330.
Benzoate, sodium, and growth, 354.
  sodium, in diet, and growth, 858.
Benzothiazole, pharmacological reactions of, 721.
Bier's spots, meaning of, 497.
Bile diversion into vena cava and portal vein, 303.
  salts in urine, test for, 209.
Bilirubin in urine, urobilinogen concentration, 629.
Bioelectric aspects of all or none law, 838.
Blastcmyces, mannitol agar in differentiation of, 544.
Blepharisma, cannibalism in, 335.
Blood, acid-base status, and parenteral fluids, 374.
  acidity, and high oxygen pressures, 832.
  and meat diet, 324.
  and tissue lactates, and muscular exercise, 292.
  and urine, levulose determination in, 248.
  and urine, ovarian hormone in, and menstrual cycle, 785.
  bacteriology in arthritis, 6.
  cells, phagocytosis of, and tannin, 876.
  changes in pH, and sodium cyanide, 829.
  chemistry, post operative, 17.
  cholesterol in, 336.
  citrated, erythrocyte sedimentation rate, and plasma proteins, 115.
  determination of acid-base balance, 780.
  during pregnancy, amino-acids, urea and non-protein nitrogen in, 804.
  effect of digitalis on, 524.
  filtrates, analysis of, 353.
  flow during periodic ventilation, 828.
  flow, glomerular, and splanchnic nerves, 792.
  flow velocity, and posture, 697.
  ion concentration changes affecting reflex time, 188.
  lactic acid content in trypanosome infection, 229.
  lipoid and inorganic phosphorus, 360.
  mechanical fragility of, 733.
  of mother and new born, 814.
  oxidation of lactic acid by erythrocytes, 865.
  phosphates in milk fever, 387.
  platelets and neoarsphenamin, 407.
  portal injection of insulin, and hyperglycemia, 485.
  pressure affected by "Vasopressin," "Oxytocin," Pituitary Extract, 243.
  pressure in skeletal muscles, and epinephrine, 472.
  regeneration in anemia, 510.
  sugar during glycolysis, 122.
  sugar estimation, 351.
  sugar utilization and epinephrine, 345.
Botulinum toxin destroyed by B. subtilis, 343.
  susceptibility to, 89.
Brain, mid, lesions causing hypothermia, 48.
  respiratory quotient, 496.
Bronchi and intestine, and ephedrin, 16.
Brucella abortus, dissociation of, 101.

Calcification of tissue, and X-Ray, 257.
Calcium action on vertebrate heart, 506.
  and phosphorus balances during pregnancy and lactation, 104.
  in hyperparathyroidism, 717.
  retention and magnesium sulfate, 58.
  and potassium on ephedrine, 680.
  blood, and tetany after thyroparathyroidectomy, 210.
  gluconate as therapeutic agent, 773.
  pilocarpine and atropine, 512.
  reserve in carbon tetrachloride and chloroform poisoning, 138.
  serum, increase by irradiated ergosterol, 199.
Calorogenic activity of suprarenal gland, 296.
Cancer and nutrition, 574.
  and tuberculosis, pathological relation, 73.
Cannibalism in Blepharisma, 335.
Carbon tetrachloride and chloroform poisoning, guanidine retention and calcium reserve, 138.
Cardiovascular response to adrenalin, 259.
Caries, dental, and mouth acidity, 718.
Cartilage, effect of heat in transplantation, 238.
Caseinogen, phosphorised, precipitin production, 222.
Castration, effect on oviduct, 652.
Cell count of peritoneal fluid, 690.
  division rate and thyroid, 231.
Cerebro-spinal fluid, reducing reaction, 78.
Chemicals, and heart rhythm, 816.
  ferrous sulphate, copper sulphate and arsenic, and vitamin A-free diet with iron, 141.
Chemo-therapeutic substances, and surface reactions, 789.
"Chilies," and digestive functions and metabolism, 273.
Chloremia, hypo, experimental, 874.
Chloroform and carbon tetrachloride poisoning, guanidine retention and calcium reserve, 138.
  pyridine test for, 403.
Cholecystokinin preparations and vaso-depressor phenomenon, 309.
Cholesterol as digitonid, determination of, 518.
  in blood, 336.
  sensitizing effect of, and ergosterol, 677.
  ultra-violet irradiation, and growth of tubercle bacilli in vitro, 43.
Ciliary body, columnar epithelium of, 739.
  currents and transportation of spermatozoa, 52.
Circulation, peripheral, and "tobacco angina," 9.
Cl. Botulinum Type D Sp. N., 88.
CO_2 and oxygen on flow of cerebro-spinal fluid, 831.
  transportation by paraffine oil, 590.
Coagulant, anti,—action of heparin in vivo, and dosage, 383.
Cocci, gram structure, 364.
Coccidioides immitis in lesions, 534.
Cod liver oil and anemia, 418.
  and fertility and incubation, 615.
Colloidal state of antiseptics and arsenicals, 790.
Complement fixation test in yellow fever, 846.
Conditioned motor responses, 342.
Conduction rate and threshold in myelinated nerves, 241.
Conductivity of protozoan cultures, 605.
Copper, effect on liver, 200.
  sulphate, ferrous sulphate and arsenic, and vitamin A-free diet with iron, 141.

- **Coracidium** of oriental diphyllobothrids, infection with, 250.
- **Corydalis** ambigua, alkaloids from, 735.
- **Culture** media preparation for pathogenic amebas, 245.
- **Curare,** effect of alkali injections, 182.
- **Currents,** high frequency, histologic effect of, 588.
- **Cyanide,** sodium, and pH of blood, 829.
- **Cystine** in sweat of cystinurics, 69.

- **Demethoxylation** of lignin, 320.
- **Depressor,** vaso,—phenomenon, and cholecystokinin preparations, 309.
- **Development** of elastic fibers in embryonic heart muscle, 779.
  - of sympathetic nervous system, 824.
- **Diabetes** and nitrogen metabolism, 181.
  - hyperglycemia of, 120.
  - phloridzin, and vital staining, 809.
- **Diabetics,** latent tolerance in, 449.
- **Diarrhea** and gastro-intestinal auto-disinfection, 159.
- **Diet,** and hemoglobin concentration in nursing young, 603.
  - and phospholipid fatty acids, 793.
  - containing sodium benzoate, and growth, 354, 858.
  - fat, influencing growth, 427.
  - meat, effect on blood, 324.
  - and X-Rays, 30.
- **Digestive** functions and metabolism, and "chilies," 273.
- **Digitalis,** circulatory changes following, 524
- **Digitonid,** cholesterol as, determination of, 518.
- **Diphtheria** toxins and antitoxins, use of paramecia, 213.
  - toxin, purification by adsorption, 696.
- **Diphyllobothrids,** Oriental, infection with, 250.
  - Oriental, sparganum stage, infection with, 252.
- **Disinfectants** and yeasts, 154.
  - resistance of purified bacteriophage to, 880.
- **Disinfection,** gastro-intestinal auto—, and diarrhea, 159.
- **Duration** of life, properties of Na, K, Mg, and Ca ions, 797.
- **Dysentery,** meta, bacilli, 545.

- **Ear** lesions and body temperature, 106.
- **Eclampsia,** and guanidine-like substances, 607.
  - hepatic lesion in, 239.
- **Ectoparasites** in Bartonella infection, 157.
- **Edema** and morphine, 572.
  - experimental, 173.
- **Egg**-waters, echinid, and surface potential difference of sperm, 392.
- **Electrocardiogram** and hypertension roentgenogram, changes in, 367.
- **Encephalitis,** herpetic, experimental, 699.
  - vaccinal, and testicular virus, 559.
- **Endocarditis,** pathogenesis of, 516.
- **Ephedrin** on intestine and bronchi, 16.
  - epinephrine antagonism, 500.
- **Epinephrin,** and reaction to pollen and histamine, 611.
- **Electric** currents and arteries, 166.
- **Electrocardiogram** voltage, and altering position of heart, 204.
  - voltage, and venous inflow to heart, 202.
- **Electrolytes** of saliva, 93.
  - penetration of, 192.
- **Electrophoresis** of B. phaseoli sojense, S. and R., 299.
- **Enamel,** vitality of dental, 69.
- **Encephalitis** herpetic, and tissue-vaccine, 24.
  - experimental herpetic, and herpes virus, 21.
- **Endothelium,** stimulation on phagocytic activity of, 294.
- **Ephedrine** homologs and isomers, pharmacological action and chemical constitution, 348.
  - potassium and calcium on, 680.
- **Epinephrine** and blood sugar utilization, 345.
  - ephedrine antagonism, 500.
  - influencing blood pressure in skeletal muscles, 472.
  - kidney and spleen response to, 443.
- **Epithelioid** cells produced in lymph glands by non-tuberculous substance, 333.
  - cells produced in subcutaneous tissues by injection of irritants, 331.
- **Epithelium,** columnar, of ciliary body, 739.
- **Ergosterol,** and increase in serum calcium, 199.
  - and sensitizing effect of cholesterol, 677.
  - irradiated, and parathyroids, 555.
  - irradiated, and thrombocyte counts, 639.
- **Ergot** alkaloids, on intestine and uterus, 501.
- **Erythrocyte,** glutathione oxidation in, 541.
  - oxidation of lactic acid by, 865.
  - sedimentation rate and plasma proteins, 115.
- **"Eucupin"** in local analgesia, toxicity of, 526.
- **Excretion** of fixed acid and base, 376.
- **Exophthalmia,** 63.
- **Eye** positions, compensatory in complete Torticollis, 81.

- **Fat,** effect of whale oil, 704.
  - food, influence on constitution of phospholipids, 436.
  - free rations, wheat germ oil, influence on fertility, 185.
  - in diet influencing growth, 427.
  - iodine values of, 515.
- **Fatigue** of muscle, and adrenalin, 136.
  - contracture and ions, 165.
- **Fatty** acids, volatile, determination of, 108.
- **Fertility,** ultraviolet light, solar irradiation and cod-liver oil, 615.
  - wheat germ oil and fat free rations, 185.
- **Fever,** milk, blood phosphates in, 387.
- **Fibrinogen,** source of, 490.
- **Fibroblasts,** classification of, 580.
- **Filtrates,** culture of skin reactivity to, 207.
- **Filtration,** ultra, studies on bacteriophage, 530.
- **Food-poisoning,** experimental, with paratyphoid bacilli, 301.
  - poisoning with paratyphoid poisons, 91.
  - irradiated, and superheating, 453.
- **Fowl**-pox by mosquitoes, 128.
- **Freemartin** effect in parabiotic twins, 655.

- **Galactose** and glucose, metabolism of, 694.
- **Gall bladder** and faradic stimulation of stomach, 466.
  - emptying, 312.
  - evacuation, and histamine, 310.
  - tonus changes by Faradic stimulation, 314.
- **Gall, ox,-** and foreign protein and insulin, 65.
- **Gastric juice,** total loss of, 305.
  - motility and insulin, 769.
  - motor mechanism, and acids, 624.
  - secretion, "basal," mechanical factors, 270.
  - intestinal auto-disinfection, and diarrhea, 159.
  - intestinal tract motility in rickets, 758.
- **Gelatin,** isoelectric point, 702.
  - serum gels orientation of cylindroid particles in, 147.
- **Gels,** gelatin-serum, 147.
- **Globin,** preparation of, 741.
- **Glucose** absorption and vitamin B deficiency, 347.
  - absorption from intestine, 433.
  - and galactose, metabolism of, 694.
  - fate of, in eviscerated animal, 432.
- **Glutathione** oxidation in erythrocytes, 541.

Glycemia, hyper, and vasopressin, oxytocin and pituitrin, 146.
  hyper, following portal injection of insulin, 485.
  hyper, of diabetes, 120.
  hypo and insulin and protein metabolism, 631.
  hypo, in nursing young with Vitamin B deficiency, 72.
Glycine, X-Ray diffraction spectra of, 527.
Glycogen content of muscles in tetanus, 466.
Glycolysis and blood sugar, 122.
Goiter and iodine, 263.
  produced by vegetables, 822.
Grafts, ovarian, and ovariectomy, 660.
Growth, and anemia treated by liver, 869.
  and diet containing sodium benzoate, 858.
  and diet on phospholipid fatty acids, 793.
  and liver extract, 862.
  and reproduction on vitamin C free diet, 276.
  and sodium benzoate, 354.
  and vaccine virus, 556.
  differentiation and proliferation in vitro, 585.
  factors in liver, 715.
  influence of fat on, 427.
  promoting power of digestive mucosa, 503.
Guanidine-like substances in liver injury and eclampsia, 607.
  retention in carbon tetrachloride and chloroform poisoning, 138.

Heart, altering position, and electrocardiogram, 204.
  and chemical control of pulmonary ventilation, 833.
  changes in respiratory and cardiac rhythms, 834.
  experimental arrhythmias, 570.
  muscle, embryonic, development of elastic fibers, 779.
  of race horse, 549.
  rhythm, effect of lithium, ammonium, strontium and barium ions, 816.
  venous inflow, and voltage of electrocardiogram, 202.
  vertebrate, action of calcium on, 506.
Heat regulation and lymph production, 169.
  resistance of B. subtilis spores, 95.
Hemagglutination by tumor extracts, 134.
Hemoglobin concentration, influence of diet on, 603.
  regeneration, and whole wheat, 322.
  standards, 848.
Hemorrhagic anemia and radiant energy, 750.
Heparin in vivo, anticoagulant action, 383.
  purified, anticomplementary action of, 508.
Heredity, intensity of, and change of sex, 851.
Herpes febrilis virus, resistance of causative agent of tumor, 480.
  virus and experimental herpetic encephalitis, 21.
  virus, and experimental immunization, 19.
Herpetic encephalitis, experimental, 699.
Hexose-phosphate formation by muscle extract, and amylase, 356.
Histamine, absorption from intestine, 765.
  absorption from intestine, and parathyroidectomy, 768.
  and pollen, skin reaction to, 611.
  dilator effect on cerebral vessels, 706.
  on gall-bladder evacuation, 310.
  skin reaction to, and epinephrin, 612.
  test for achlorhydria during fever, 422.
Histologic effect of high frequency currents, 588.
Histolysis, tail skin, during metamorphosis, 451.
Hormone, anterior hypophyseal, and hyperplasia of mammary apparatus, 598.
  maturity, and hyperplasia of mammary apparatus, 597.
  maturity, stimulation of placentoma reaction by, 597.
  and birth mechanism, 595.
  pituitary on thyroid, 860.
  male, 325.
  male, 569.
  seasonal variation in interstitial tissue of testis, 727.
  standardization of male, 686.
  follicular, 685.
  follicular, and basal metabolism, 265.
  œstrus producing, chemistry of, 569.
  oestrous, in urine, 586.
  ovarian, and basal metabolism, 782.
  ovarian, content of urine in pregnancy, 882.
  assay in blood and urine, and menstrual cycle, 785.
  inducing menstruation, 430.
  and hen-feathering, 86.
Hydrion concentration, sap, and mineral nutrient balance, 189.
Hydroquinone in vitamin A deficiency, 328.
Hydroxyl, base excretion, fixed acid and, 376.
Hyperplasia of mammary apparatus by anterior hypophyseal maturity hormone, 597.
  of mammary apparatus induced by anterior hypophyseal hormones, 598.
Hypophyseal hormones, hyperplasia of mammary apparatus, 598.
  maturity hormone, and hyperplasia mammary apparatus, 597.
  maturity hormone, stimulation of placentoma, 597.
Hyphophysis, cell types in pars anterior, 424.
  maturity hormone, and birth mechanism, 595.
Hypothermia due to lesions in midbrain, 48.

Icteric index and Van den Bergh tests, 438.
Immune horse serum, typhus bacillus, 267.
Immunity, local, in lungs immunized against pneumococci, 314.
  ultra-violet absorption related to, 878.
  virus, in tissue cultures, 494.
  to B. sordellii, maternally transmitted, 799.
Immunization by type specific pneumococcic pleural exudates, 687.
  experimental, with herpes virus, 19.
  oral and pneumococci, 565.
Inanition, and phospholipid fatty acids, 795.
Indole, bactericidal action, 391.
Infection, acute of respiratory tract, 562.
  resistance, and vaccination with pneumococcus, 226.
  with poliomyelitis virus, 632.
  with sparganum stage of Oriental Diphyllobothrids, 252.
  and inadequate supply of Vitamin A, 233.
  and thyroid, 472.
Inheritance, and hypersensitiveness, 679.
  of respiratory anaphylaxis, 8.
Inoculation, intratracheal, and lung abscess, 163.
Insulin activity, inhibition of, 11.
  and foreign protein after ox-gall, 65.
  and phosphocreatine, 533.
  and protein metabolism, 631.
  isolation in crystalline form, 219.
  on gastric motility, 769.
  portal injection, and hyperglycemia, 485.
Interference and circuit transmission of activation waves, 316.
Interstitial tissue of testis, seasonal variations, 727.
Intestine obstruction, experimental, 464.
  absorption of histamine from, 765.
  and bronchi, and ephedrin, 16.
  and uterus, action of ergot alkaloids, 501.
  reciprocal activity of muscle coats, 813.

In vitro cultures, virus immunity in, 494.
    differentiation and proliferation, 583.
    tissues in latent life, 585.
    tubercle bacilli, and irradiated cholesterol, 43.
In vivo heparin, anticoagulant action, 383.
Iodine and goiter, 263.
    inorganic, injection and metamorphosis, 191.
    values of rat fat, 515.
    oils, on serous membranes, 307.
Ion concentration of blood, reflex time, 188.
    and fatigue contracture, 165.
    of adsorbed protein, 689.
Iron, vitamin A-free diet, and chemicals, 141.
    iodide to vitamin A-free diet, and arsenic, 143.
Irradiated ergosterol, and parathyroids, 555.
    ergosterol and thrombocyte counts, 639.
    foods, antirachitic properties influenced by superheating, 453.
Irradiation, and potentiometric and spectrophotometric changes, 618.
    of cholesterol, and growth of tubercle bacilli in vitro, 43.
    of crude coal tar, 33.
    serum calcium increase and ergosterol, 199.
    solar, and fertility and incubation, 615.
Isoelectric point of gelatin, 702.

Jaundice, activity of stomach during, 770.
Joint cavities, lymphatic drainage, 395.

Kala-azar, transmission of, and sandflies, 277.
    transmitted by sandflies, and slapping, 280.
KI and anterior pituitary, and thyroid gland, 109.
Kidney and spleen response to epinephrin, 443.
    enlargement, high concentrations of protein, and vitamins B and G, 725.

Labyrinth destruction and body temperature, 104.
Lactates, and muscular exercise, 292.
Lactic acid content of blood and trypanosome infection, 229.
    increase in muscle under anesthetics, 641.
    oxidation by erythrocytes, 865.
Lactation and pregnancy, C and P balances, 104.
Latent life, tissues in vitro, 585.
Leptospira icterohaemorrhagiae, 26.
Lesions, haemorrhagic glomerular, and Streptococcus viridans, 397.
Leucocytes, and Cathode rays, 380.
Leukemia, experimental transmission, 362.
Levulose determination in blood and urine, 248.
    tolerance for, in experimental liver injury, 249.
Lignin, demethoxylation of, 320.
Lipin content of nervous system in anesthesia, 776.
    phosphorus determination, 67.
Lipoid and inorganic phosphorus in blood, 360.
Liver, and adrenalin and pituitrin, 628.
    autolysis in vivo, 304.
    effect of copper, 200.
    extract on food consumption, utilization and growth, 862.
    extract standardization, 13.
    growth factors in, 715.
    in anemia, and basal metabolism, 872.
    in anemia, and growth, 869.
    injury and eclampsia, and guanidine-like substances, 607.
    injury, experimental, and tolerance for levulose, 249.
    lesions in eclampsia, 239.
Locke's for perfusion of segments of intestine, 811.
Luminous substances, stability of, 133.
Lung abscess, chronic, production of, 28.
Lutaum, corpus, inhibiting oestrus, 818.
Lymph glands, epithelioid cells in, by injection of non-tuberculous substance, 333.
    production and heat regulation, 169.
    drainage of joint cavities, 395.
Magnesium sulfate in retention of Ca. and P., 58.
Malaria, anemia from, and reticulocyte behavior, 417.
Mannitol agar in differentiation of blastomyces, 544.
    fermenting monilia, 544.
Measles, toxins and antitoxins, use of paramecia, 213.
Meningeal blood vessel, and afferent nerve fibers, 288.
Meninges, permeability of, and hyperpyrexia, 286.
Menstrual cycle and ovarian hormone in blood and urine, 785.
Menstruation induced by ovarian hormone, 430.
Metabolism, thyroid substance and KI, 636.
    and digestive functions, and "chilies", 273.
    and urine pentose, 321.
    basal, and injection of follicular hormone, 265.
    basal, and liver in anemia, 872.
    basal, and ovarian hormone, 782.
    basal, and skin temperature, 784.
    basal, and thyroid, 389.
    nitrogen, and diabetes, 181.
    of galactose and glucose, 694.
    protein, and insulin, 631.
Metals combined with urease or coenzymes of amoebocytes, 111.
Metamorphosis, atrophy in, 650.
    by inorganic iodine injection, 191.
    influence of atrophying gills, 183.
    integumentary pigmentation during, 451.
Methylene blue inactivating staphylococcus bacteriophage, 100.
Microorganisms, skin reactivity to culture filtrates, 207.
Monilia, mannitol fermenting, 544.
Monocytes produced in subcutaneous tissues by irritants, 331.
    reaction in Virus III disease, 666.
    reaction in neoplasia, 668.
    response to peroxydase test, 55.
Morphine, anesthesia and pupillary reactions, 3.
    and edema, 572.
    and feeding in conditioned salivary response, 167.
    salivary conditioned response, extinction of, 168.
Mosquitoes and fowl-pox, 128.
Mucosa, digestive, growth-promoting power, 503.
Muscle coats of intestine, reciprocal activity of, 813.
    extract, formation of hexose-phosphate, and amylase, 356.
    in tetanus, glycogen content of, 466.
    skeletal, blood pressure in, and epinephrine, 472.
    contraction and oxygen, 830.
    exercise, and lactates, 292.

Neoplasia, monocytic reaction in, 668.
Nephritis, streptococcal, urinary findings in, 546.
Nerve fibers, afferent, in meningeal blood vessel, 288.
    unmyelinated fibers, 349.
    and pupillary dilatation, 461.
    extrinsic, degeneration of, and reflex arcs, 867.

  myelinated, threshold and conduction rate, 241.
  somatic, action potential in fibers, 646.
  splanchnic, on glomerular blood flow and reflex aneuria, 792.
  system, differentiation of fibers, 645.
  system in anesthesia, lipin content of, 776.
  system, sympathetic, development of, 824.
Nicotine tolerance, 845.
Nitroprusside, toxicity of, 102.
Nutrient balance, mineral, and sap hydrion concentration, 189.
Nutrition and cancer, 574.
  mineral, and isolation, 662.

Oestrus and reflex time, 657.
  hormone in urine, 586.
  inducing effect of taurocholate, 820.
  inhibited by extracts of corpus luteum, 818.
  producing hormone, chemistry of, 568.
Oil, paraffine, transportation of CO_2 by, 590.
  whale and depot fat, 704.
  wheat germ, fat free rations, and fertility, 185.
  iodized, on serous membranes, 307.
Osmotic pressure, colloid, and serum proteins relations, 1.
Ouabain and barium chloride, and ventricular fibrillation, 201.
Ovariectomy and transplantation in pregnancy, 659.
  differential reaction to ovarian grafts, 660.
  early, spermatogenesis following, 338.
Ovary, and urine in pregnancy, 720.
Oviduct, effect of castration, 652.
Oxygen and blood flow, 828.
  and CO_2 on flow of cerebro-spinal fluid, 831.
  and muscular contraction, 830.
  and response to reflex and motor stimulation, 826.
  and response to reflex stimulation, 827.
  peripheral chemical control of pulmonary ventilation, 833.
  pressures, blood acidity, blood flow and respiration, 832.
Oxyhemoglobin determination by Duboscq colorimeter, 791.
"Oxytocin", effect on blood pressure, 243.
  vasopressin, and pituitrin, and hyperglycemia, 146.

Pancreatectomy, effect of, 463.
Paramecia for studying toxins and antitoxins, 213.
  thirteen thousand generations, 707.
Parasiticidal potencies of derivatives of rosanilin, 748.
Parathyroidectomy, and absorption of histamine from intestine, 768.
Parathyroids and irradiated ergosterol, 555.
  hyper, calcium and phosphorus in, 717.
Paratyphoid bacilli, experimental food poisoning with, 301.
  intoxication, experimental, 49.
  poisons, heat stabile, and food, 91.
Pars anterior of hypophysis cell types in, 424.
Pathogenic properties of organisms in arthritis, 863.
Penetration of alkaloids, 135.
  of dyes into living cells, prediction of, 125.
  of electrolytes, 192.
Pentose, urine, in normal metabolism, 321.
Peristalsis, intestinal, and urethane, 871.
Peritoneal fluid, cell count of, 690.
Peritonitis, typhoid, and bacteriophage, 753.
Permeability into living cells, 290.
  of dyes, into living cells, prediction of, 125.
  of electrolytes, 192.
  of meninges, and hyperpyrexia, 286.
  penetration of alkaloids, 135.
  resistance changes in collodion membranes and cells, 358.
Peroxydase test, monocytic response to, 55.
Perspiration and sleep, 577.
Phagocytic activity of endothelium, stimulation on, 294.
Phagocytosis of blood cells, and tannin, 876.
Pharmacological action, chemical constitution of ephedrine homologs and isomers, 348.
  reactions of benzothiazole, 721.
Phosphates, blood, in milk fever, 387.
Phosphoric acid, tertiary dissociation constant, 129.
Phosphorus and calcium in hyperparathyroidism, 717.
  and C balances during pregnancy and lactation, 104.
  and Ca. retention and magnesium sulfate, 58.
  lipin, determination, 67.
  lipoid and inorganic in blood, 360.
Phosphocreatine and insulin, 533.
Phospholipid fatty acids, and diet, 793.
  fatty acids, and inanition, 795.
  influence of food fat on, 436.
Pilocarpine and atropine, effect of calcium on, 512.
  on pupil, 79.
Pituitary and KI, and thyroid gland, 109.
  extract, effect on blood pressure, 243.
  hormones of anterior, and thyroid, 860.
  transplants and reproduction, 692.
Pituitrin and adrenalin, and liver, 628.
  vasopressin and oxytocin, and hyperglycemia, 146.
Placentoma reaction by anterior hypophyseal maturity hormone, 597.
Plethysmograms, recording of, 711.
Pneumococci, and local immunity, 314.
  and oral immunization, 565.
  typing, agglutination, antibody determination, 492.
  pleural exudates, and immunization, 687.
  anti-, protective substance in normal serum, 284.
  intracutaneous vaccination, antibody response, 224.
  vaccination with, and hypersensitiveness, 227.
  vaccination with, and resistance to infection, 226.
Poliomyelitic virus and streptococci, resistance in glycerol, 337.
Poliomyelitis, and lesions in mucus membrane, 664.
  virus infection, 632.
  virus of, 600.
Pollen and histamine, skin reaction to, 611.
  proteins, fractionation, and skin reactions, 771.
  skin reaction to, and epinephrin, 612.
Posture, and velocity of blood flow, 697.
Potassium and calcium on ephedrine, 680.
  iodide and thyroid, 468.
Potatoes, sweet, Vitamin A and B content, 254.
Potential difference of sperm, and echinid egg-waters, 392.
Potentiometric changes by irradiation, 618.
Precipitin tests with tubercle bacilli, 672.
Pregnancy, amino-acids, urea and non-protein nitrogen in blood during, 804.
  and lactation, C and P. balances, 104.
  antitryptic titre in, 592.
  ovarian hormone content of urine during, 882.
Proteins and diazotized aromatic amines in neutral solution, 482.
  antiseptic activity of adsorbed, 787.
  denaturization rate, and specific antibodies, 525.
  foreign, and insulin after ox-gall, 65.

  intake, effect on reproduction, 521.
  ionization of adsorbed, 689.
  plasma, erythrocyte sedimentation rate, 115.
  renal enlargement, and vitamins B and G, 725.
  solutions concentrated without denaturation, 744.
Protoplasm, living, reversible changes, 124.
Protozoan cultures, conductivity of, 605.
Pulmonary suppuration, pathogenesis of, 723.
Pupil, and pilocarpine, 79.
  and sympathomimetic drugs, 80.
  dilatation, reciprocal innervation during, 461.
  reactions during anesthesia after morphine, 3.
Pyrexia hyper on permeability of meninges, 286.
Pyridine test for chloroform, 403.

Quinine salts solubility, and alkalis, 613.

Rachitic, anti, properties of irradiated foods, and superheating, 453.
Radiation, on anemia, 751.
  on hemorrhagic anemia, 750.
  resistance to tetanus toxin in rickets, 757.
  infra-red, and rickets, 77.
  on S. aureus and B. coli communis, 14.
  ultra-violet absorption, and immunity, 878.
  ultra-violet, and fertility and incubation, 615.
  ultraviolet, and stimulation and inhibition, 175.
  ultraviolet transparency of human skin, 778.
  motility of gastro-intestinal tract in rickets, 758.
  cathode, and leucocytes, 380.
  secondary, on S. aureus and B. coli communis, 14.
Recorder, automatic drop, 52.
"Refection," 552.
Reflex and motor stimulation response, and low oxygen, 826.
  aneuria mechanism, and splanchnic nerves, 792.
  arcs, enteric and degeneration of extrinsic nerves, 867.
  cutaneous in hypothyroidism, 776.
  response, and sodium sulphide and cyanide, 825.
  stimulation, response to, and respiration, 828.
  stimulation, response to, and ventilation, 827.
  time and changes in ion concentration of blood, 188.
  time and oestrus, 657.
  conditioned, 81, 342.
  extinction of morphine salivary conditioned response, 168.
  morphine and feeding in salivary response, 167.
Regeneration, blood, in anemia, 510.
  hemoglobin, and whole wheat, 322.
Reproduction and growth on vitamin C free diet, 276.
  effect of protein intake on, 521.
Resistance and basophilia, 670.
  of purified bacteriophages to disinfectants, 880.
Respiration, irregular, and response to reflex stimulation, 828.
  tissue, and thyroidectomy, 439.
Respiratory anaphylaxis following sensitization, 127.
  anaphylaxis, sensitizing dose, 325.
  and cardiac rhythms, synchronous experimental changes in, 834.
  depressants on respiratory quotients of excised tissue, 722.
  quotient of brain, 496.
  quotients of excised tissue, and respiratory depressants, 722.
  tract, acute infection, 562.
Reticulocyte behavior in anemias, 417.
Reversible changes in living protoplasm, 124.
Rickets and infra-red radiation, 77.
  and radiation on resistance to tetanus toxin, 757.
  endogenous, 181.
Rinderpest, experimental, 22.

Salicylate, sodium, and skin allergy, 857.
Saliva, electrolytes of, 93.
  gland poison of Aedes aegypti, 806.
  slapping in transmission of kala-azar, 280.
Sandflies, and transmission of kala-azar, 277.
Sap hydrion concentration and mineral nutrient balance, 189.
Scarlet fever streptococcus toxin, 50.
  toxins and antitoxins, use of paramecia, 213.
Schick toxin, buffered diluent for, 801.
Secretion, "basal" gastric, 270.
Sensitiveness, hyper, and vaccination with pneumococcus, 227.
Serum, immune, and B. typhosus filtrates, 131.
  antipneumococcus protective substance, 284.
  calcium increase by irradiated ergosterol, 199.
  gelatin-, gels, 147.
  death following injection, 117.
  proteins and colloid osmotic pressure relations, 1.
Sex change and intensity of heredity, 851.
Skin reactions and fractionation of pollen proteins, 771.
  reactivity to culture filtrates, 207.
  ultraviolet transparency of, 778.
Sleep and insensible perspiration, 577.
Sodium iso-amyl-ethyl barbiturate inducing anesthesia, 399.
  ricinoleate, and subcutaneous tissue, 854.
  sulphide and cyanide on reflex response, 825.
Spectrophotometric changes by irradiation, 618.
Speech, air and bone transmitted, quantitative testing, 355.
Sperm, surface potential difference, and echinid egg-waters, 392.
  transportation and ciliary currents, 52.
Spermatogenesis following ovariotomy, 338.
Spinach and anemia, 835.
Spinal fluid, cerebro-, reducing reaction, 78.
  fluid, rate of flow, and low oxygen and high alveolar CO$_2$, 831.
Spirochete, in vitro cultivation, 761.
Spleen and kidney response to epinephrin, 443.
  changes in size after exercise, 529.
  denervated, in adrenalectomy, 271.
  etiology of splenomegaly, 410.
  filaments in siderotic nodules, in splenomegaly, 413.
Splenomegaly, etiology of, 410.
Staphylococcus aureus and B. coli communis, radiation on, 14.
  bacteriophage inactivated by methylene blue, 100.
Stimulation, and phagocytic activity of endothelium, 294.
  in Nitella, 836.
Stomach activity in jaundice, 770.
  faradic stimulation, and gall-bladder, 466.
  motility and asthenia, 621.
Streptococcus and poliomyelitic virus, resistance in glycerol, 337.
  hemolyticus, dissociation, 145.
  toxin, scarlet fever, 50.
  viridans, and haemorrhagic glomerular lesions, 397.

Streptococcal nephritis, urinary findings in, 546.
Succinoxidase activity of tissues and thyroidectomy, 441.
Sugar, blood, during glycolysis, 122.
  blood, estimation of, 351.
  blood, utilization and epinephrine, 345.
  fate of, in eviscerated animal, 432.
Suprarenal gland, calorogenic activity of, 296.
Susceptibility to Botulinum toxin type B, C, D, 89.
Sweat of cystinurics, cystine in, 69.
Sympathetic irritability and hyperthyroidism, 459.
Sympathomimetic drugs on pupil, 80.
Synergism, bacterial, on endo medium, 41.
  hemolytic bacterial, 205.
Syphilis test, sensitizing effect of cholesterol, and ergosterol, 677.

Tar, crude coal, irradiation, 33.
Taurocholate inducing oestrus, 820.
Tea and Vitamin C, 82.
Teeth and thyroparathyroidectomy, 598.
Temperature, body, and lesions of vestibular nuclei, 106.
  critical, and thermal increments of biological reactions, 446.
  regulation, and labyrinth destruction, 104.
  skin and basal metabolism, 784.
Tension, hyper, roentgenogram and electrocardiogram, changes in, 367.
Testis, arterial supply of, 178.
Tetanus, glycogen content of muscles in, 466.
  and blood calcium after thyroparathyroidectomy, 210.
Thyroid after KI and anterior pituitary, 109.
  and basal metabolism, 389.
  and hormones of anterior pituitary, 860.
  and infections, 472.
  and potassium iodide, 468.
  and rate of cell division, 231.
  and sympathetic irritability, 459.
  anterior lobe substance, and precocious metamorphosis, 149.
  anterior lobe substance, effect of feeding, 151.
  anterior lobe substance in absence of thyroid gland, 153.
  effect of anterior lobe substance on, 152.
  hyper, antitryptic titre in, 592.
  hypo, experimental, reflex in, 776.
  transplantation into brain, 236.
  substance and potassium iodide on metabolic rate, 636.
Thyroidectomy and succinoxidase activity of tissues, 441.
  and tissue respiration, 439.
Thyroparathyroidectomy and teeth, 598.
  tetany and blood calcium, 210.
Tomatoes, Vitamin A during ripening, 45.
Tonus changes in gall bladder by Faradic stimulation, 314.
Torticollis, compensatory eye positions in, 81.
Toxicity of nitroprusside, 102.
Toxin, Botulinum, destroyed by B. subtilis, 343.
  botulinum, susceptibility to, 89.
  and antitoxins, use of paramecia, 213.
  and venoms, effect of, 522.
  bacterial, purification by adsorption, 696.
  heat labile, in closed intestinal loops, 654.
  scarlet fever streptococcus, 50.
  Schick, buffered diluent for, 801.
Transplants, adrenal in transparent chamber, 745.
Transmission and interference of activation waves, 316.
  of leukemia, experimental, 362.
  speech, air and bone, quantitative testing, 355.
Transplantation and ovariectomy in pregnancy, 659.
  effect of graded degrees of heat upon cartilage, 238.
  of skin bacteria, 113.
  of mammalian tissues, 372.
  of thyroid gland into brain, 236.
  of tissues, 731.
  eyes, correlation in development of structures associated with, 726.
  eyes, return of vision, 477.
  limbs, movements in, 729.
  of pituitary, and reproduction, 692.
Trimethyl derivatives of rosanilin, parasiticidal potencies of, 748.
Trypanosome infection, and lactic acid content of blood, 229.
Trypsin susceptible bacteriophage adapted to trypsin, 97.
Tubercle bacilli, 456.
  precipitin tests with, 672.
  in vitro, and cholesterol irradiated by ultra-violet, 43.
Tuberculosis and cancer, pathological relation, 73.
Tuberculous, non-, infections of urinary tract, bacteria in, 499.
  non-, substance producing epithelioid cells in lymph glands, 333.
Tularemia, experimental, 260, 426.
  natural infection, 626.
Tumor extracts, hemagglutination by, 134.
  resistance of causative agent to solvents compared with vaccine virus and Herpes febrilis virus, 480.
Twins, parabiotic, freemartin effect, 655.
Typhoid peritonitis, and bacteriophage, 753.
Typhus bacillus immune serum, 267.
Tyrode's for perfusion of segments of intestine, 811.

Urea concentration test, 40.
Urease of amoebocytes combined with metals, 111.
Uremia and enteritis, 171.
Uretero-ureteral anastomosis, 18.
Urethane and intestinal peristalsis, 871.
Urine findings and renal pathology in streptococcal nephritis, 546.
  tract, bacteria in non-tuberculous infections of, 499.
  and blood, levulose determination in, 248.
  and blood, ovarian hormone in, and menstrual cycle, 785.
  containing bilirubin, urobilinogen concentration in, 629.
  in pregnancy, and ovary, 720.
  in pregnancy, ovarian hormone content, 882.
  oestrous hormone in, 586.
  pentose in normal metabolism, 321.
  test for bile salts, 209.
Urobilinogen in urine containing bilirubin, 629.
Uterus and intestine, action of ergot alkaloids, 501.

Vaccine encephalitis and testicular virus, 559.
  intracutaneous, with pneumococcus, antibody response, 224.
  with pneumococcus, and hypersensitiveness, 227.
  with pneumococcus, and resistance to infection, 226.
  in experimental herpetic encephalitis, 24.
  virus in growing embryo, 556.
  virus, resistance of causative agent of tumor, 480.
Van den Bergh tests and icteric index, 438.
"Vasopressin," effect on blood pressure, 243.
  oxytocin and pituitrin, and hyperglycemia, 146.
Vegetables and goiter, 822.
Velocity of blood flow, and posture, 697.

Venoms and toxins, effect of, 522.
  dried, crystalline principles, 378.
Ventricular fibrillation, effect of barium chloride and ouabain, 201.
Vibriothrix, new, 543.
Virulence of B. phaseoli sojense, S. and R, 299.
Virus, effect of drying on, 479.
  immunity in tissue cultures, 494.
  of Avian molluscum, filtration of, 217.
  of poliomyelitis, 600.
  poliomyelitis, infection, 632.
  testicular, and vaccinal encephalitis, 559.
  III disease, monocytic reaction, 666.
  vaccine, in growing embryo, 556.
  vaccine, resistance of causative agent of tumor, 480.

Vital staining and phloridzin diabetes, 809.
  stains, penetration into living cells, 290.
Vitamin A and B content of sweet potatoes, 254.
  A deficiency, and hydroquinone, 328.
  A during ripening of tomatoes, 45.
  A-free diet with iron, and arsenic, copper sulphate and ferrous sulphate, 141.
  A, inadequate supply, and acute infections, 233.
  B and G, relation to renal enlargement, and protein, 725.
  B content of sweet potatoes, 254.
  B deficiency and absorption of glucose, 347.
  B deficiency, and hypoglycemia in nursing young, 72.
  B, heat-stable factor in, 536.
  C free diet, and growth and reproduction, 276.
  C in tea, 82.
  E, sources of, 521.
  G, heat-stable factor, 536.

Wheat, whole, and hemoglobin regeneration, 322.

X-Ray diffraction spectra of glycine, 527.
  investigations on calcification of tissue, 257.
  relation of dietary, 30.

Yeasts and disinfectants, 154.

E. V. McCOLLUM

3 8198 313 601 484
THE UNIVERSITY OF ILLINOIS AT CHICAGO